CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

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MICROBIOLOGY REVIEW(S)

MICROBIOLOGY REVIEW
DIVISION OF SPECIAL PATHOGEN AND TRANSPLANT PRODUCTS

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(Original)

REVIEWERS: Aaron Ruhland and Simone M. Shurland
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SPONSOR: Novartis Pharmaceuticals Corporation
One Health Plaza, Bldg. 405/4051
East Hanover, NJ 07936-1080

DRUG CATEGORY: Anti-malarial

INDICATION: Treatment of *Plasmodium falciparum* malaria

DOSAGE FORM: Tablets for oral administration

PRODUCT NAMES:

a. PROPRIETARY: Coartem

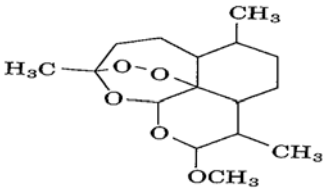
b. NONPROPRIETARY: Artemether and lumefantrine (benflumentol)

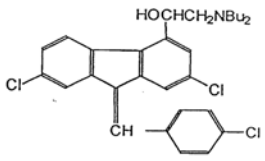
c. CHEMICAL:

Artemether: (3R, 5aS, 6R, 8aS, 9R, 10S, 12R, 12aR)-Decahydro-10-methoxy-2,6,9-trimethyl-3,12-epoxy-12H-pyrano(3,2-j)-1,2-benzodioxepin

Lumefantrine: (±)-2-Dibutylamino-1-(2,7-dichloro-9-(4-chlorobenzyldiene)-9H-fluoren-4yl)-ethanol

STRUCTURAL FORMULA:





	Artemether	Lumefantrine
Molecular weight:	298.4.	528.9
Molecular formula:	$C_{16}H_{25}O_5$	$C_{30}H_{32}C_{13}NO$

SUPPORTING DOCUMENTS: None.

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1. EXECUTIVE SUMMARY

Nonclinical Microbiology:
Mechanism of action
Coartem is a fixed dose combination of artemether and lumefantrine in the ratio of 1:6. Artemether is rapidly metabolized into the active metabolite dihydroartemisinin (DHA). Both artemether and DHA are sesquiterpenes with an endoperoxide moiety. The anti-malarial activity of artemether and DHA has been attributed to its endoperoxide moiety. The exact mechanism by which lumefantrine exerts its anti-malarial effect is not well defined. Available data suggest lumefantrine inhibits the formation of β-hematin by forming a complex with hemin. Both artemether and lumefantrine were shown to inhibit nucleic acid and protein synthesis.

Activity *in vitro*
The activity of artemether, DHA, and lumefantrine was measured against several laboratory strains and clinical isolates from Thailand, Africa, China, Philippines, and French Guiana as measured by incorporation of ^{3}H–hypoxanthine or by microscopic method. The results, expressed as 50% and 90% inhibitory concentration (IC_{50} and $IC_{90,}$ respectively) values, show that artemether, DHA, and lumefantrine are active against the erythrocytic stages of *P. falciparum.* Artemether IC_{50} values were similar to DHA.

Combination of artemether with lumefantrine in the ratio of 10:1 and 1:100 was tested against 3 strains of *P. falciparum* (K1, T-996, and LS-21). Results, expressed as IC_{50} and IC_{90} values, show that a combination of artemether with lumefantrine to be 3 to 100 fold more active than either drug alone.

Activity *in vivo*
The activity *in vivo* was measured against the **erythrocytic stages** of *P. berghei*, *P. knowlesi,* and *P. falciparum* strains in either mice or monkeys.

Mice infected with the N strain of *P. berghei* and treated at time of infection with lumefantrine or artemether (n=5 per group) showed a 50% reduction in parasitemia at doses of 1.27 mg/kg and 2.7 mg/kg, respectively. The time required for reducing the parasitemia by 50% was 2 times faster in mice treated with artemether (mean, 23 hours) compared to that of lumefantrine (mean, 54 hours). Treatment with lumefantrine resulted in clearance of parasitemia; whereas treatment with artemether often resulted in recrudescence of infection.

A combination of artemether and lumefantrine in a ratio of 1:0.375 resulted in a rapid reduction in parasitemia similar to that of artemether alone; clearance of parasitemia was similar to that of lumefantrine alone.

Monkeys (n=3 per group) infected with *P. knowlesi* and treated with artemether alone showed a faster reduction in parasitemia but did not clear the parasites. Treatment with lumefantrine alone showed a slower reduction in parasitemia; however, most animals were aparasitemic on day 105. A combination of artemether and lumefantrine (in the ratio of either 1:4 or 1:6) was more effective in a faster reduction of parasitemia and clearance of parasites from blood in all animals than either drug alone.

Similar results were observed in monkeys infected with *P. falciparum.* There appears to be no antagonism between artemether and lumefantrine.

Drug Resistance

In vitro studies in which the erythrocytic forms of *P. falciparum* K1 strain were serially passaged (number of passages not specified) showed no decrease in sensitivity to artemether, lumefantrine, or the combination of artemether and lumefantrine.

The *in vitro* activity of artemether against *P. falciparum* clinical isolates from French Guiana measured between 1997 and 2005 showed a decrease in the *in vitro* sensitivity to artemether in 2002 and 2005. Nine of the isolates in 2002 and 1 isolate in 2005 had an IC_{50} greater than 8.9 ng/mL. Molecular typing indicated that these isolates had a *PfATPase*6 –S769N mutant allele. Re-culture of the stored isolates with the mutant allele *PFATPase* 6-S769N in the absence of artemether for 3-weeks showed a decrease in IC_{50} value (1.42 ng/mL), suggesting a poor fitness of the mutant allele.

In vivo studies from mice infected with *P. berghei* strains showed that the potential to develop resistance to artemether, lumefantrine and a combination of artemether+ lumefantrine exists. A study also showed resistance to artemether may be unstable often resulting with the reversal to a more sensitive strain. Clinical relevance of such an effect is not known.

Clinical Microbiology

The applicant has submitted 24 clinical studies supporting the efficacy of Coartem in the treatment of uncomplicated falciparum malaria in China, Thailand, Kenya, Nigeria, Tanzania, Mozambique, Mali, Republic of Benin as well as non-immune travelers from Germany, Switzerland and Colombia. Of the 24 studies, datasets were available for 8 clinical studies. The parasitological evaluations were performed using Giemsa stained thick and/or thin smears. The study protocols specified that parasite density/μL be based on actual patient WBC counts. However, site inspection by the Division of Scientific Investigation (DSI) revealed that at some sites the parasite density/μL was based on a WBC count of 8000/μL (for details see DSI report). Such a difference in calculations should not affect the efficacy and parasitologic findings as long as consistency is maintained.

Coartem was nearly 100% effective in accomplishing microscopically confirmed parasite clearance within 7 days of treatment with a median parasite clearance time (PCT) of 34 hours. Parasites subsequently reappeared in some patients by day 28. Irrespective of the dose and regimen of Coartem, the overall cure rates across all studies in the intent to treat (ITT) and per protocol (PP) populations were approximately 89% and 97%, respectively. Gametocyte counts were performed in each trial and results show that although most patients who presented with gametocytemia at baseline were free of gametocytes by day 7, gametocyte clearance time was longer than the asexual PCT. However, some patients who presented with gametocytes at baseline remained positive for gametocytes until their last examination.

The sponsor has presented the 28 day cure rates as corrected cure rates, based on genotyping, for 6 clinical studies in the proposed package insert. Genotyping was done to differentiate recrudescence

from a new infection. The applicant has utilized two different polymerase chain reaction (PCR) techniques, in 2 different laboratories. The PCR assay performed at the Shoklo Malaria Research Unit was used to analyze samples from Studies 025, 026, and 028. Samples from Studies 2401, 2403 and 2303 were tested by PCR and restriction fragment length polymorphism assays at the (b) (4) However, due to a lack of performance characteristics of the assay and quality control, the results of these analyses should be interpreted with caution and only uncorrected cure rates should be used in determining the efficacy of Coartem.

Most (n=12) of the remaining 16 clinical studies were a 4-dose regimen studies and did not achieve high cure rates. There were 3 studies supporting the 6-dose regimen of Coartem. The 28 day uncorrected cure rates (>90 %) were available for two of the three 6-dose regimen studies conducted in Vietnam and Bangladesh and support efficacy of Coartem in the treatment of falciparum malaria. Datasets for all the 16 studies were not available for review

2. INTRODUCTION AND BACKGROUND

The subject of this NDA is Coartem (20 mg artemether / 120 mg lumefantrine) tablets for the treatment of acute uncomplicated malaria due to infections with *Plasmodium falciparum* or mixed infections including *P. falciparum*. The applicant has proposed a combined oral formulation of two compounds - artemether (20 mg) and lumefantrine (120 mg) in a fixed combination (ratio 1:6) for a total of 6 doses over 3 days. The treatment schedule includes an initial dose followed by a second dose after 8 hours and then twice daily (morning and evening) for the following two days.

Artemether is the semi-synthetic beta-methylether derivative of the natural artemisinin, obtained from the Chinese herb *Artemisia annua*. Artemether is a sesquiterpene lactone containing an endoperoxide bridge. Artemether is rapidly metabolized into an active metabolite dihydroartemisinin (DHA). Lumefantrine is a fluorine derivative, synthesized in 1970s. Normal synthesis of lumefantrine yields the racemate of the two stereo-enantiomers, lumefantrine (+) and lumefantrine (–)[1].

After administration of the fixed combination (6-dose/3 days) regimen to adult patients with falciparum malaria, the peak plasma concentrations of artemether, DHA, and lumefantrine occurred at 2, 2 and 6-8 hours, respectively (Table 1). In healthy subjects the lumefantrine half life was 3 to 6 days. For details see Clinical Pharmacology review.

Table 1: Summary of the mean PK parameters of lumefantrine, artemether and DHA in adult malaria patients

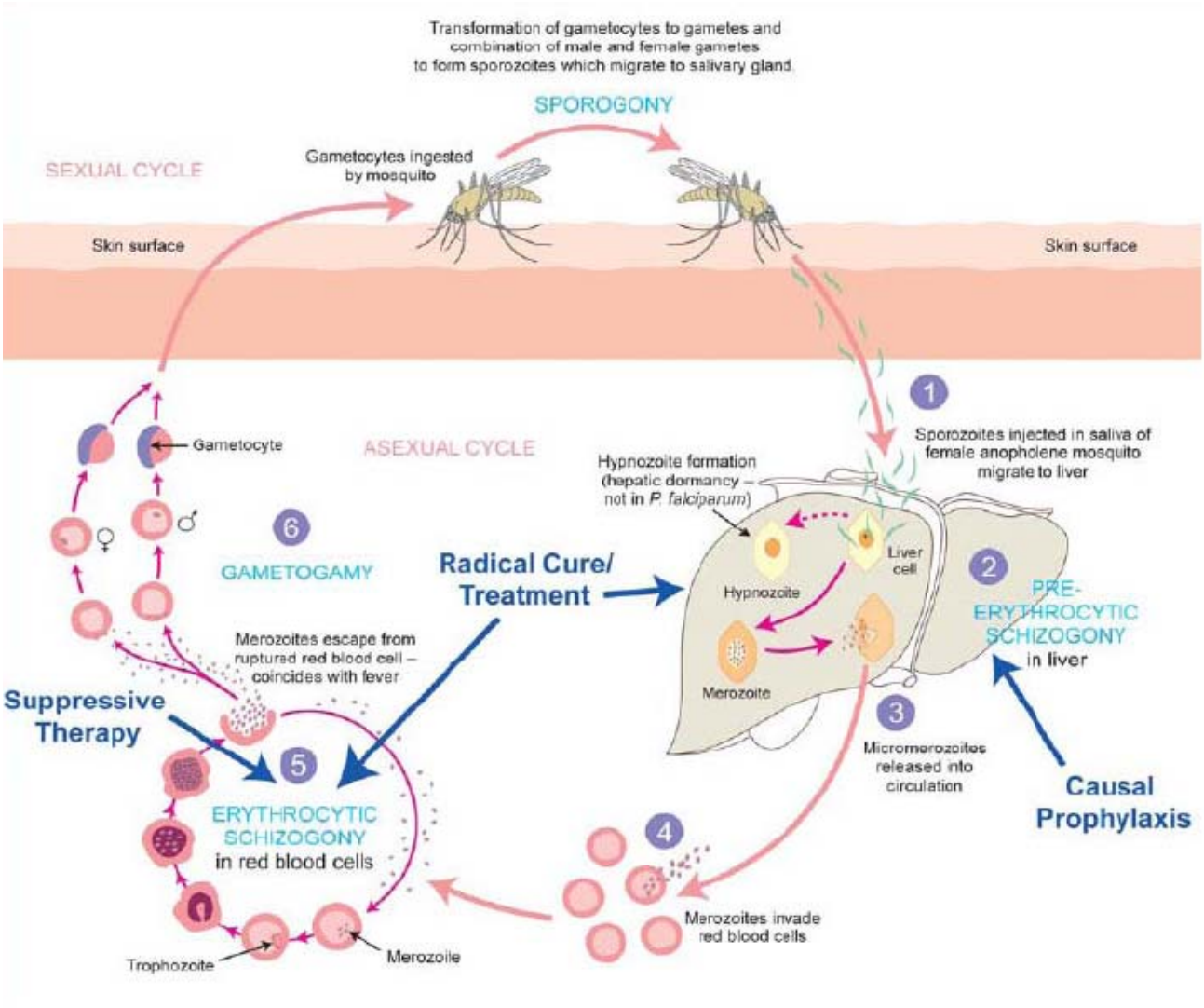
Analyte	Mean C_{max}	Mean AUC_{last}	Mean T_{max} (hours)	$T_{½}$ (hours)
Lumefantrine	5.72 - 25.7 µg/mL	272 - 758 µg/mL	60-62	ND
Artemether	66.2 - 186 ng/mL	211 - 535 ng/mL	2	1.6 -2.2
DHA	101 - 205 ng/mL	320 - 604 ng/mL	2 – 3	1.5 -1.6

ND = not determined.

2.1 Biology of the parasite

The four *Plasmodium* species responsible for human malaria are *P. falciparum, P. malariae, P. vivax* and *P. ovale.* The life cycle of all the *Plasmodium* species involves both sexual reproduction (**sporogony**) and asexual reproduction (**schizogony**). The sexual cycle including sporogony occurs in the mosquito and the asexual cycle including pre-erythrocytic schizogony and erythrocytic schizogony occurs in the human host (Figure 1).

Figure 1: Life Cycle of malaria (Reproduced with modification by permission of Health Protection Agency, United Kingdom)[2]



The cycle in the human host is initiated when a malaria-infected female *Anopheles* mosquito injects infective **sporozoites** into the human host bloodstream during a blood meal. The sporozoites are thought to leave the bloodstream and infect liver cells (sometimes referred to as **exo-erythrocytic**

schizogony stage). Maturation of the parasites into **schizonts** in the liver may take 5 to 15 days depending on the species. Rupturing of the mature schizont from the liver and release of **merozoites** into the blood stream signal the beginning of the erythrocytic phase. Generally, only one cycle of merozoite production occurs in the liver before red cells are invaded. For *P. vivax* and *P. ovale*, parasites can persist in the liver as a dormant stage, referred to as **hypnozoites**, and cause relapses, by invading the bloodstream weeks, or even years later. Neither *P. falciparum* nor *P. malariae* has a persistent liver phase. In the case of falciparum malaria, the recurrence of malaria is due to incomplete elimination of the blood stage of the parasite either due to inadequate treatment of blood schizonticides or to waning immunity and is termed **recrudescence.**

After initial replication in the liver, merozoites have structures that selectively adhere to the erythrocyte membrane or attach to receptors on the membrane. *P. vivax* uses receptors for specific blood group antigens such as in the Duffy system, whereas *P. falciparum* attaches to receptors on the erythrocyte membrane. Once merozoites have attached, they initiate the **erythrocytic phase** of infection (or the **erythrocytic schizogony** stage). Within the erythrocyte, the parasite feeds on hemoglobin and goes through a maturation sequence asexually marked with distinct morphology such as the **ring stage trophozoite** to **mature trophozoite.** Malarial pigment is formed in the growing trophozoite as a result of incomplete metabolism of the hemoglobin referred to as **hemozoin.** The mature trophozoite (**schizont**) begins to divide, ruptures and releases a new wave of merozoites that invade a new batch of erythrocytes and repeat development into a schizont. The release of generations of merozoites from erythrocytes, malarial metabolites and endotoxin-like substances into the bloodstream prior to their invasion of new erythrocytes is responsible for the clinical manifestations of the disease (chills and fever).

The merozoites begin differentiating into sexual erythrocytic stages (**gametocytes**) presumably when the environment of the host becomes hostile. The gametocytes, male (**microgametocytes**) and female (**macrogametocytes**), circulate in the bloodstream and are ingested by the female *Anopheles* mosquito during a blood meal.

The parasites' multiplication in the mosquito is known as the **sporogonic** cycle. While in the mosquito's stomach, fertilization occurs in which the microgametocytes penetrate the macrogametocytes and develop into **gametes.** The resulting **zygotes** in turn become motile and develop into elongated **ookinetes.** The ookinetes migrate through the stomach wall of the mosquito where they develop into **oocysts**. The oocysts grow to maturity until they rupture, and release thousands of **sporozoites**. The sporozoites make their way to the mosquito's salivary glands.

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3. PRECLINICAL/NONCLINICAL MICROBIOLOGY

3.1 MECHANISM OF ACTION

Coartem is a fixed dose combination of artemether and lumefantrine in the ratio of 1:6.

3.1.1 Artemether and DHA

The anti-malarial activity of artemether and DHA has been attributed to an endoperoxide bridge moiety. The effect of artemether and/or DHA on protein alkylation, oxidative stress, in metabolic processes such as glycolysis, nucleic acid and protein synthesis, in the presence or absence of compounds such as heme and iron were examined.

3.1.1.1 *Uptake of DHA or artemether by erythrocytes*

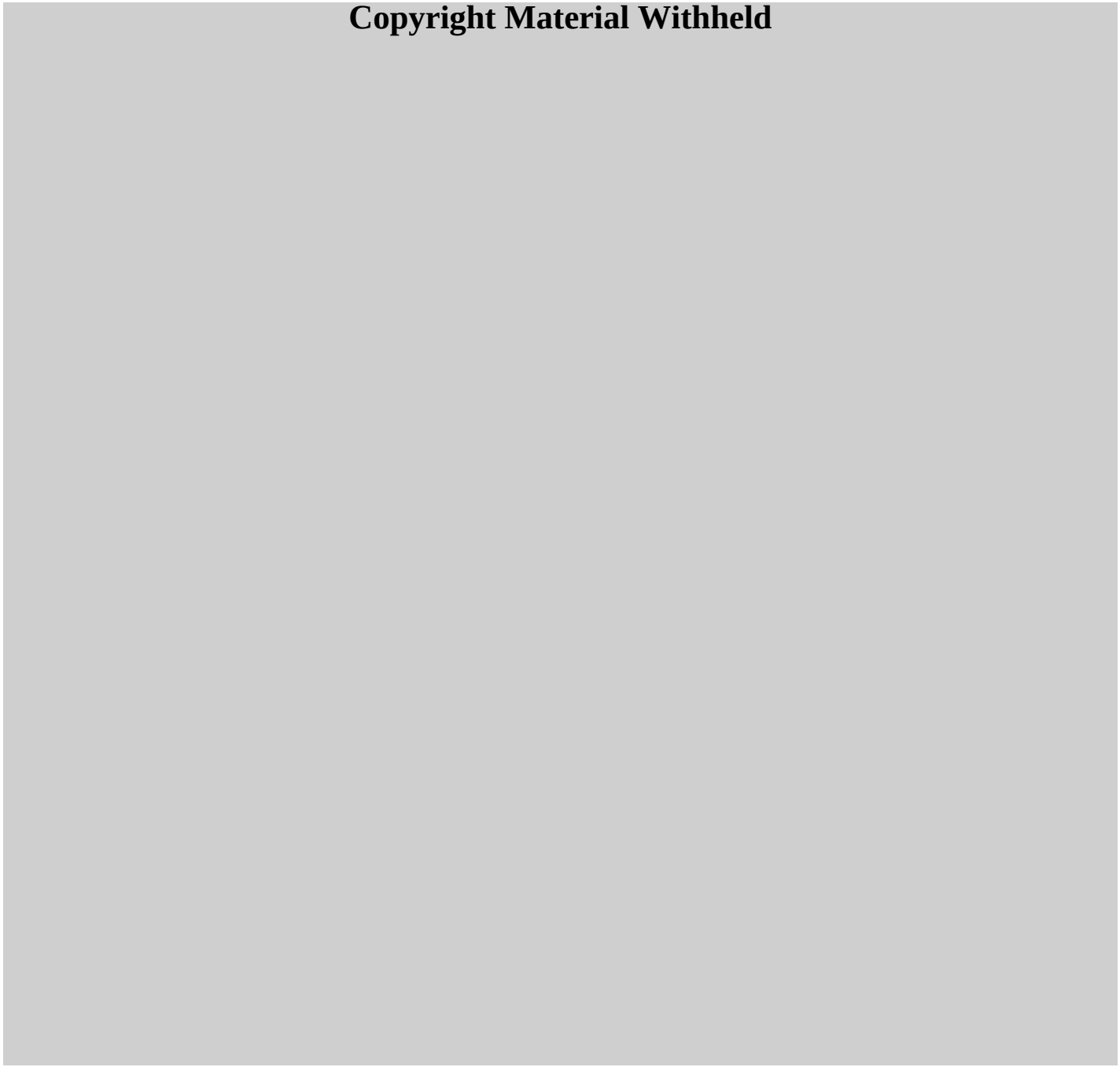
The uptake of radiolabeled DHA (^{3}H- DHA) in erythrocytes infected by *P. falciparum* (Wellcome/Liverpool/West African strain) was examined *in vitro*[3]. Parasitized erythrocytes in RPMI medium with 10% human serum were incubated with ^{3}H- DHA (12 nM i.e. 3.40 ng/mL) at 37°C for up to 20 hours. Uninfected erythrocytes were used as control groups. An aliquot of the cultures was collected and the radioactivity was measured at different time points (up to 20 hours). The results in Figure 2 show the maximum uptake ratio of ^{3}H- DHA in uninfected erythrocytes was 1.7 after 3 hours incubation and remained at this steady level even up to 20 hours. In parasitized erythrocytes uptake of ^{3}H- DHA was faster and much higher than the uninfected erythrocytes. The maximum uptake of ^{3}H- DHA in infected erythrocytes containing different developmental forms (57% small ring stage, 29% large ring stage + trophozoites stage , 8% pre-schizont stage, and 6% schizont stage) occurred by 2 hours .The uptake gradually declined by 20 hours of incubation.

Figure 2: Time-course of uptake over 20 hour of ^{3}H- DHA by *P. falciparum* infected erythrocytes

In another experiment, the uptake of ^{3}H- DHA was measured *in vitro* using 2 different concentrations of parasitized erythrocytes (8% and 10%). Total and differential parasite counts of the developmental forms (i.e. small ring, large ring mature trophozoite, pre-schizonts and schizonts) were carried out using light microscopy. Uninfected erythrocytes were used as control groups. The results in Figure 3 and Table 2 show that the uptake of ^{3}H- DHA was higher in infected erythrocytes (Figure 3B and 3C)

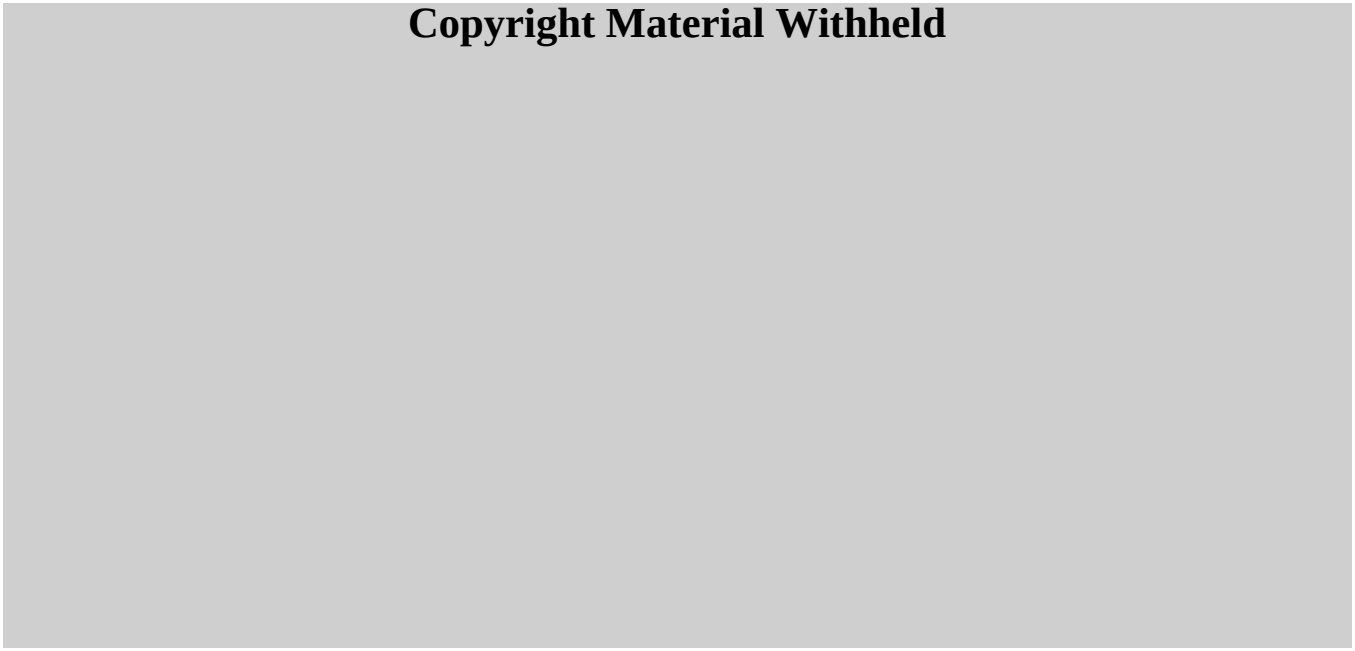
compared to uninfected erythrocytes (Figure 3A). In the parasitized erythrocytes that contained early stage developmental forms (Figure 3B) the uptake of ^{3}H- DHA was slower compared to parasitized erythrocytes that contain late stage developmental forms (Figure 3C). However, after 2 hours of incubation, the uptake of ^{3}H- DHA by the parasitized erythrocytes was similar in the cultures (10 %) which contained early developmental forms compared to the cultures (8%) with higher numbers of late developmental forms (Figure 3 and Table 2).

Figure 3: Time-course of uptake over 20 hour of ^{3}H- DHA by uninfected and erythrocytes infected with *P. falciparum*



In another experiment, the effect of artemether, DHA, chloroquine, and anisomycin (protein synthesis inhibitor) on the uptake of ^{3}H- DHA was examined. As in the time course experiment described above, parasitized erythrocytes were exposed to ^{3}H- DHA (12 nM i.e. 3.40 ng/mL) and incubated at 37°C. The addition of 3 μM concentrations of non-radioactive drugs (i.e. artemether, DHA, chloroquine and anisomycin) were added either 1 hour before, at the same time, or 1 hour after the addition of ^{3}H- DHA. Uptake of ^{3}H- DHA alone was used as a comparator. The results in Figure 4-I show that the uptake of ^{3}H- DHA was greatly inhibited by artemether and DHA when added before or simultaneously; chloroquine was less effective. However, addition of non-radioactive drugs one hour after the addition of ^{3}H- DHA showed a marked effect only with artemether and DHA but not with chloroquine. The results in Figure 4-II showed that anisomycin showed little effect on DHA uptake.

Figure 4: Effects of artemether (AE), dihydroartemisinin (DHA), chloroquine (CQ), and anisomycin (An) at 3 μM concentrations on uptake of ^{3}H- DHA by erythrocytes infected with *P. falciparum*



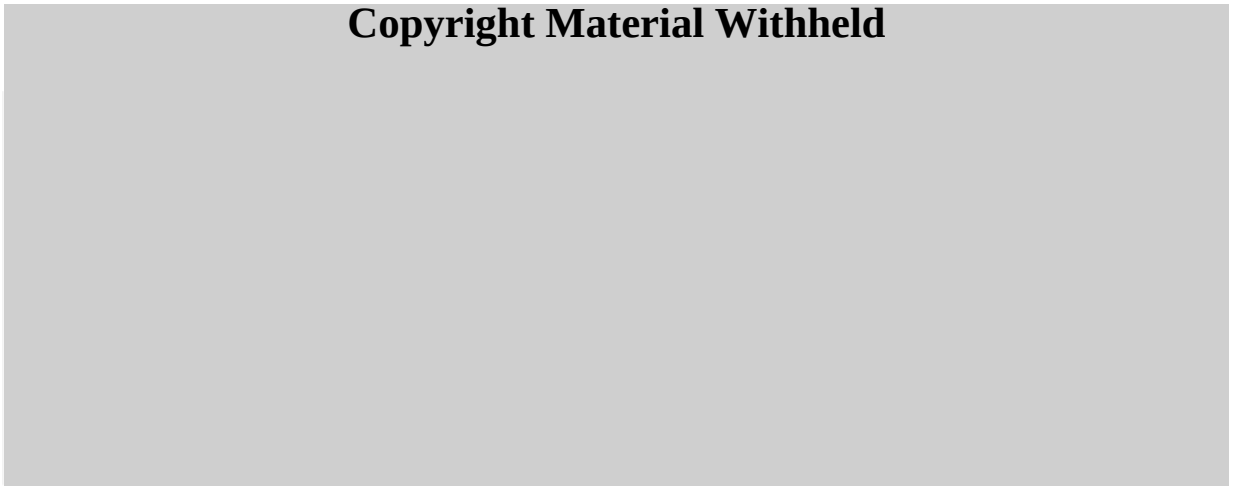
3.1.1.2 *Role in protein alkylation*

In a study by Yang *et al.,*[4] the alkylation of human serum by DHA, in the presence of thiol and/or amine blocking agents was examined *in vitro* using ^{3}H- DHA. Human serum was pre-incubated with thiol blocking agents, 30 nM iodoacetamide (IA) or 10 nM N-ethylmalemide (NEM) for 20 minutes. An aliquot of iodoacetamide treated serum was also incubated with an amine blocking agent, 30 mg/ml of succinic anhydride (SA). Unbound serum protein was separated by gel filtration using a NAP-5 column. Serum proteins, with the amine and sulfahydryl groups blocked, were treated with ^{3}H- DHA and incubated at 37°C. Untreated serum proteins with the amine and sulfahydryl groups blocked served as comparators. At timed intervals of 0, 2, 6 and 24 hours, samples were gel filtrated using a NAP-5 column to remove unbound ^{3}H- DHA. Radiolabeled thiol content was quantified using a scintillation counter. The results in Figure 5 show that there was an increase in ^{3}H- DHA bound to untreated serum proteins over time. It was stated that after 24 hours 17 – 20% of DHA was bound to

serum proteins. In the presence of thiol blocking agents (IA and NEM) the binding of ^{3}H- DHA was reduced by 15% and 58%, respectively. There was a greater inhibitory effect with IA and SA than with IA alone. The greater inhibitory effect also seen with NEM could be a reflection that NEM blocks both SH and amino groups. The study suggests that DHA alkylates human serum by reacting with thiol and amino groups. It is not known whether artemether has similar alkylating properties.

Figure 5: Effects of IA, NEM and IA + SA pretreatment on ^{3}H- DHA binding to serum

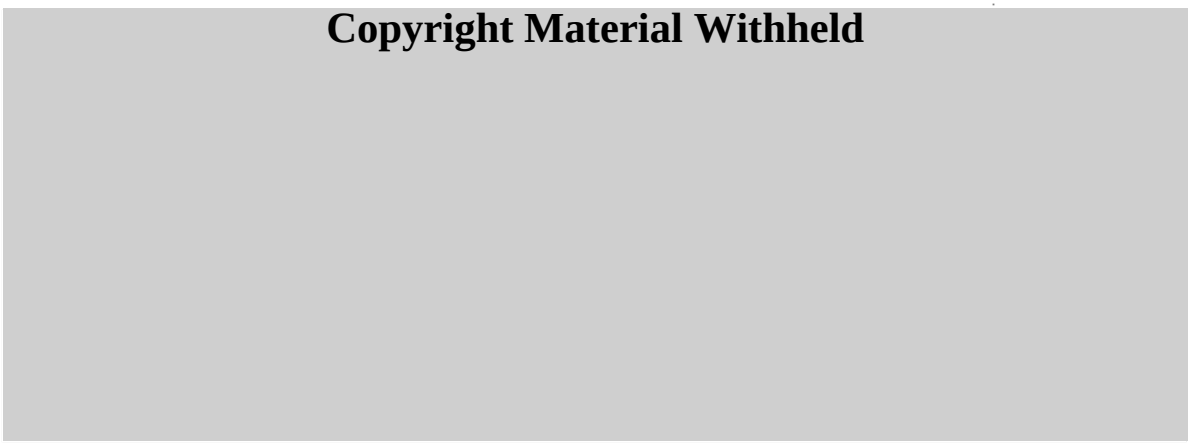
In another experiment, the alkylation of human serum proteins incubated with ^{3}H- DHA in the absence or presence of hemin and/or denaturing agents was examined. Human serum proteins and ^{3}H-DHA in the absence or presence of hemin were incubated for 1 hour at room temperature in the presence of denaturing agents, *5%* mercaptoethanol or 8M urea. The proteins were separated according to their electrophoretic mobility by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS- PAGE). The results of the autoradiograph (Figure 6) show that the drug bound protein (68 kDa) was similar in size to that of the human serum albumin (66 kDa). The intensity of the radioactive band (Figure 6) of drug bound protein was similar to the drug bound protein when treated with mercaptoethanol or urea. The proteins from the gel were fractionated by electrospray ionization – mass spectra (ESI-MS). The ESI-MS showed that bound albumin with artemisin had a higher mass spectrophotometry weight i.e., M_r (67,223 ± 34) than albumin alone (M_r = 66, 745 ± 35). The results suggest that DHA binds to human serum protein covalently.

Figure 6: 

3.1.1.3 *Role of artemether in oxidative stress*

In a study by Senok *et al.*,[5] the effect of artemether against β-thalassemic erythrocytes in the presence of riboflavin (pro-oxidant) and vitamin E (anti-oxidant) was examined. Normal erythrocytes were used as comparators. Synchronization of two *P. falciparum* (FC27 and K1) strains was carried out using sorbitol. The effect of artemether in combination with Vitamin E or riboflavin was measured using parasitized whole blood. Synchronized cultures at the early young ring and trophozoite erythrocytic stages were added to artemether coated wells and cultures incubated for 12 hours at 37°C. In the presence of artemether, there was no significant difference in the level of parasitemia in the β-thalassemic erythrocytic cultures or normal erythrocytic cultures (Figure 7). The addition of vitamin E significantly reduced the effect of artemether against both parasite strains in normal and thalassemic erythrocytes; whereas the addition of riboflavin significantly increased the effect of artemether against both parasite strains in normal and thalassemic erythrocytes compared to artemether alone.

Figure 7: Effect of artemether in the presence of Vitamin E and riboflavin against FC27 (A) and K1 (B) strains of *P. falciparum*



3.1.1.4 *Effect on nucleic acid and protein synthesis and glycolysis*

The effect of artemether on protein and nucleic acid synthesis as well as on glycolysis in cultures of *P. falciparum* K1 strain was examined[6]. For the effect on nucleic acid synthesis, the trophozoite stages of *P. falciparum* K1 strain were incubated in the presence of ^{3}H-hypoxanthine, the nucleic acid precursor, for 30 minutes before the drugs were added. For the effect on protein synthesis, the protein precursor L-(4,5-^{3}H)-isoleucine was added. For the effect on glycolysis, the production of lactic acid was measured. Parasitized erythrocytes were then incubated with artemether at 10 times the IC_{50} value. The cells were collected at 30-minute interval up to 3.5 hours and the radioactivity measured. Drug-free cultures were used as controls. The results in Figure 8 show artemether inhibited nucleic acid synthesis and protein synthesis. The authors state that artemether had no effect on lactate production in glycolysis; however, no data were shown.

Figure 8: Effect of artemether on nucleic acid (A) and protein synthesis (B) in *P. falciparum* K1 strain

3.1.2 Lumefantrine

The ability of lumefantrine to bind with heme, and its effect on hematin, nucleic acid synthesis, protein synthesis, and glycolysis were examined.

3.1.2.1 *Effect on nucleic acid and protein synthesis as well as glycolysis*

The effect of lumefantrine on protein and nucleic acid synthesis as well as on glycolysis was examined[6] using cultures of *P. falciparum* K1 strain as described above in section 3.1.1.4. The results in Figure 8 show that lumefantrine, like artemether, inhibits nucleic acid and protein synthesis The authors state that lumefantrine had no effect on glycolytic lactate production; however, no data were included.

3.1.2.2 *Binding with hemin and β-hematin*

The ability of lumefantrine to bind with haemin (oxidized heme) and inhibit β-hematin formation was examined[6]. The binding of haemin with lumefantrine and other anti-malarial agents as positive controls was detected by a color change using toluene-soluble complexes. The inhibition of β-hematin formation was measured spectrophotometrically using haemin chloride. The formation of a complex of lumefantrine with haemin was not detected in the aqueous or organic phase (Table 3). The formation of a complex between lumefantrine and haemin led to the loss of the typical ultraviolet absorption peak of β-hematin at 1660 and 1210 nm indicating the inhibition of the formation of β-hematin.

Table 3**:** Effect of lumefantrine and other anti-malarials on the binding of haemin and inhibition of β-hematin formation.

3.2 ACTIVITY *IN VITRO*

The *in vivo* activity of artemether, DHA, and lumefantrine alone or in combination was measured against the erythrocytic forms of *P. falciparum* either by microscopic method or by incorporation of ^{3}H-hypoxanthine.

Cultures examined by the light microscopy method were counted following 48 hour incubation at 37°C and 95% humidity in an airtight candle jar containing 5% O_2, 5% CO_2, and 90% N_2. The supernatant was removed and thin blood-smeared films were made from the pellet in each well, stained with Giemsa and counted under the light microscope. The results were expressed as effective concentration values and represent the concentration at which the drug inhibited schizont maturation by 50%, 90% and 99%, (EC_{50}, EC_{90}, and EC_{99}, respectively) compared to the drug free control.

Cultures labeled with ^{3}H-hypoxanthine were incubated with ^{3}H-hypoxanthine at 37°C under micro-aerophilic conditions for 48 hours. The plates were then harvested on to glass filter mats and counted using a scintillation counter. The results were expressed as the drug concentration at which the growth of the parasite via incorporation of ^{3}H-hypoxanthine in the presence of drug was inhibited by 50%, 90% and 99% (IC_{50}, IC_{90} and IC_{99} values, respectively) as compared to drug free control wells.

3.2.1 *In vitro* activity against the erythrocytic stages of laboratory strains

3.2.1.1. Artemether

The activity of artemether against two *P. falciparum* strains, K1 and 3D7, was measured by growth inhibition in a continuous culture system[6]. The results expressed as EC_{50} value showed that strain K1 was a multidrug resistant strain including chloroquine resistance and 3D7 was sensitive to most anti-malarial drugs. The mean (± standard deviations) artemether IC_{50} values against the *P. falciparum* K1 and 3D7 strains were 0.28 ± 0.1 nM (i.e., 0.08 ± 0.03 ng/mL) and 1.05 ± 0.37 nM (i.e.,0.31 ± 0.11 ng/mL), respectively.

Alin *et al.*,[7] measured the activity of β-artemether against two *P. falciparum* strains, T-996 and LS-21. Strain T-996 was chloroquine sensitive, mefloquine, and pyrimethamine resistant whereas LS21 was chloroquine and pyrimethamine resistant. Cultures were examined using the microscopy method. The artemether EC_{50} value was 0.28 nM (i.e., 0.083ng/mL) for the T996 strain and 0.18 nM (i.e., 0.053ng/mL) for the LS-21 strain.

In another study[8] the *in vitro* activity of artemether and other artemisinin derivatives against the *P. falciparum* F32 (Tanzania), 7G8 (Brazil), Honduras (Honduras), and D7 (Tanzania) strains was measured. Strains F32, 7G8 and Honduras were chloroquine sensitive and D7 was chloroquine resistant. Cultures were examined using the microscopic method. Against the chloroquine resistant strain D7, artemether was more sensitive (mean EC_{50}, 0.98 nM i.e., 0.29 ng/mL) than the chloroquine sensitive strains F32, 7G8 and Honduras (range mean EC_{50}, 3.2 – 6.1 nM i.e., 0.95 – 1.82 ng/mL). Most of the strains were more sensitive to artemether compared to artemisinin (Table 4).

Table 4: Activity *in vitro* for artemether, DHA and artemisinin against four strains of *P. falciparum*

In another study[9] , the *in vitro* activity of artemether and other artemisinin derivatives against the *P. falciparum* strains K1 (India), LS21 (India) and T996 (Thailand) was measured. Strains K1 and LS21

were chloroquine resistant and T996 had intermediate sensitivity to chloroquine. Cultures were examined using the light microscopy method. The chloroquine resistant strains, K1 and LS21, were more sensitive to artemether than the intermediate chloroquine resistance T996 strain (mean EC_{50} value, 1.6 nM i.e., 0.48 ng/mL) (Table 5). Artemether was 3-fold more active than artemisinin against all strains, but either similar or 2-fold more active than DHA against some of the strains.

Table 5: Activity *in vitro* to artemether and other artemisinin derivatives against 3 *P. falciparum* strains

Shmuklarsky *et al.,*[10] measured the *in vitro* activity of the anomers of artemether and other artemisinin derivatives against the *P. falciparum* D6 (Sierra Leone), W2 (Indochina) and Smith/RE (Vietnam) strains. Strain D6 was chloroquine, mefloquine and quinine sensitive, whereas W2 clone and Smith/RE strains were chloroquine and quinine resistant but mefloquine sensitive. Activity was measured by the hypoxanthine incorporation method. The results in Table 6 shows that both α-artemether and β-artemether were active against all 3 strains with IC_{50} values < 1.84 nM (i.e. 0.55 ng/mL). β-artemether was 2-fold more active than artemisinin, but DHA showed greater activity compared to all the artemisinin compounds tested against all the strains.

Table 6: *In vitro* activity of β-artemether and other artemisinin compounds against 3 *P. falciparum* strains

Basco *et al.,*[11] measured the *in vitro* activity of artemether and artemisinin derivatives against the African laboratory *P. falciparum* strains FCM 29 (Cameroon), L-3 (Cote d'Ivoire) and L-16 (Sierra Leone).). Strains L-3 and L-16 were chloroquine sensitive and FCM 29 was chloroquine resistant. Cultures were examined using the ^{3}H-hypoxanthine method. The results in Table 7 show that the chloroquine resistant strain FCM-29 was 2 to 4 times more sensitive (mean EC_{50} value, 5.97 nM i.e., 1.78 ng/mL) to artemether than the chloroquine sensitive strains L-3 [mean EC_{50} value, 8.54 nM (2.54 ng/mL)] and L-16 (mean EC_{50} value, 22.7 nM i.e., 6.77 ng/mL). Artemether was 2-fold more active than artemisinin, however, was similar in activity to other artemisinin derivatives, arteether, and artelinate against all the strains tested.

Table 7: Activity *in vitro* to artemether and standard anti-malarial agents against *P. falciparum* strains

3.2.1.2. Lumefantrine

The activity of the stereo-enantiomers of lumefantrine was measured against two *P. falciparum* strains, T-996 and LS-21[1]. Strain T-996 was chloroquine sensitive but mefloquine and pyrimethamine resistant, and LS21 was chloroquine and pyrimethamine resistant. Activity of the enantiomers of lumefantrine was tested in a continuous *in vitro* culture system at 37^{0}C for 48 hours. Cultures were examined using the light microscopy method. The results in Table 8 show that the activity of the stereo-enantiomers of lumefantrine was similar against laboratory strains of *P. falciparum.*

Table 8: Activity *in vitro* to enantiomers of lumefantrine against *P. falciparum* strains

Drug	**T-996**		**LS-21**	
	IC_{50} (nM)	**IC_{90} (nM)**	**IC_{50} (nM)**	**IC_{90} (nM)**
Lumefantrine (+)	0.9	19.86	1.02	39.18
Lumefantrine (-)	1.02	18.71	0.91	25.38

The activity of artemether against two *P. falciparum* strains, K1(multidrug resistant including chloroquine resistant) and 3D7 (sensitive to most anti-malarial drugs), was measured by incorporation of ^{3}H-hypoxanthine[6]. The mean and standard deviations lumefantrine IC_{50} values for the K1 and 3D7

strains were 116.7 ± 40.55 nM (61.7 ± 21.4 ng/mL) and 131.8 ± 43.5 nM (69.7 ± 23.0 ng/mL), respectively.

The activity of lumefantrine racemate against two *P. falciparum* strains, T-996 and LS-21 was measured[7]. Strain T-996 was chloroquine sensitive, mefloquine and pyrimethamine resistant, and LS21 was chloroquine and pyrimethamine resistant. The activity of drugs was measured by microscopic examination. The EC_{50} value for lumefantrine racemate was 2.3 nM (1.21 ng/mL) against the T996 strain and 1.9 nM (1.0 ng/mL) against the LS-21 strain.

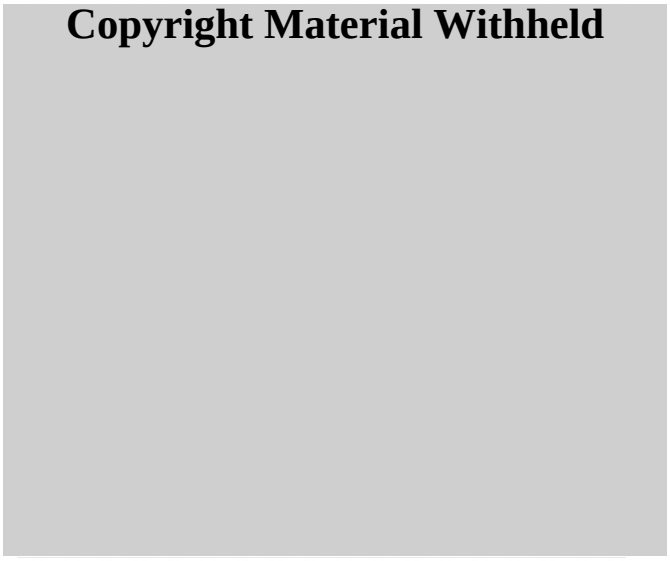
In another study[12], the *in vitro* activity of lumefantrine against synchronized ring stages of *P. falciparum* strains K1 (Thailand), 7G8 (Brazil), T9-96 (Thailand), F122 (Nigeria), F138 (Nigeria), and 160-3 (derived from the K1 strain) was measured by incorporation of ^{3}H-hypoxanthine. The results in Table 9 show an increase in sensitivity to lumefantrine against the chloroquine resistant, mefloquine sensitive laboratory strains (K1, 160-3, and 7G8), with the mean IC_{50} range between 182.7 – 200.7 nM i.e., 96 - 106 ng/ml. Against the chloroquine sensitive, mefloquine resistant strains (T9-96, F122, F138) there was a decrease in sensitivity to lumefantrine with a mean IC_{50} range of 532.3 – 684.3 nM i.e., 281.5 -361.9 ng/ml. For the laboratory strain 160-3, a K1 *P. falciparum* mutant, showed no change in sensitivity to lumefantrine 200.7 nM i.e., 106.1 ng/ml when compared to the parent K1 strain sensitivity (199.3 nM i.e., μg/ml).

Table 9. *In vitro* susceptibility of six strains of *Plasmodium falciparum* to lumefantrine and other anti-malarial agents

3.2.1.3. Combination of artemether and lumefantrine

The *in vitro* effect of artemether and lumefantrine against the *P. falciparum* K1 strain was examined by light microscopy[6]. The lumefantrine and artemether IC_{90} values were plotted as an isobologram. The results in Figure 9 suggest a synergistic effect between artemether and lumefantrine.

Figure 9. Fractional IC_{90} *in vitro* activity of lumefantrine versus artemether plotted as isobologram



In another study, the *in vitro* activity of β-artemether in combination with lumefantrine racemate mixture (at different concentrations and in different proportions) was tested against two strains of *P. falciparum*[7]. The T-996 *P. falciparum* strain from Thailand was resistant to chloroquine and pyrimethamine; whereas the LS-21 strain from India was stated to be resistant to chloroquine and with low sensitivity to pyrimethamine. Combinations of β-artemether and lumefantrine racemate mixtures over the range of 100:1 to 1:100 were tested in a continuous *in vitro* culture system at 37^0C for 48 hours. The results in Table 10 show that against the T-996 strain, the EC_{50} values for artemether alone and lumefantrine racemate alone were 0.28 nM (i.e., 0.08 ng/mL) and 2.3 nM (i.e., 1.2 ng/mL), respectively. Against the LS-21 strain, the EC_{50} values for artemether alone and lumefantrine alone were 0.18 nM (0.05 ng/mL) and 1.9 nM (i.e., 1.0 ng/mL), respectively. Based on EC_{50} values, increased sensitivity against the T-996 strain was observed for combined concentrations of β-artemether to lumefantrine racemate mixtures for the isolate between the ratios of 10:1 (0.19 nM : 0.02 nM, i.e. 0.57 ng/mL : 0.01 ng/mL) and 1:100 (0.02 nM : 2.29 nM i.e. 0.006 ng/mL : 1.21 ng/mL). Against LS-21 strains, increased activity was observed at combined concentrations of 10:1 (0.18 nM : 0.06 nM i.e. 0.05 ng/mL : 0.03 ng/mL) to 1:30 (0.05 nM : 1.52 nM i.e. 0.01 ng/mL : 0.8 ng/mL) of β-artemether to lumefantrine racemate mixtures, respectively. At the EC_{90} and EC_{99} levels, there was a 3-fold to 100-fold increase in activity as compared to the activity of β-artemether alone or lumefantrine alone.

Table 10. Inhibitory concentrations of combined mixtures of β-artemether and lumefantrine racemate in nM

3.2.2. *In vitro* activity against the erythrocytic stages from clinical isolates

3.2.2.1 *Artemether*

In a study by Bustos *et al.,*[13] the *in vitro* activity of artemether and other artemisinin derivatives was measured against 31 *P. falciparum* clinical isolates in the Philippines by the ^{3}H-hypoxanthine incorporation. The artemether IC_{50} values ranged between 0.7 and 53 nM (0.21 – 15.8 ng/mL). The results in Table 11 show that the mean EC_{50} and EC_{90} values for artemether were 3.4 nM (1.01 ng/mL) and 29.3 nM (8.74 ng/mL), respectively. The activity of other artemisinin derivatives such as arteether (range, 0.06 – 1.21 ng/mL) and artesunate (range, 0.11 – 3.15 ng/mL) against the clinical isolates was more than artemether, however, artemisin was least active of all the artemisinin derivatives. The Philippine region is known to be resistant to chloroquine and amodiaquine. Among the isolates tested, 18 % (7/38) showed high IC_{50} values for chloroquine, 8% (3/36) for mefloquine, and 5% (2/37) were high for halofantrine. A positive correlation was observed in the activity of artemether and other artemisinin derivatives as well as artemether and halofantrine ($r = 0.42$, $p = 0.018$), suggesting cross-resistance between the drugs (Table 12).

Table 11. The EC_{50} Activity *in vitro* of artemether and other standard anti-malarial drugs against the *P. falciparum* clinical isolates from the Philippines in 1991

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Table 12. Correlation between anti-malarial agents against the *P. falciparum* clinical isolates from the Philippines in 1991

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Pradines *et al.*,[14] measured the *in vitro* activity of artemether against 51 *P. falciparum* clinical isolates in Senegal, East Africa from 1995 – 1996 by incorporation of ^{3}H-hypoxanthine. The results in Table 13 show that the artemether IC_{50} value ranged between 0.8 – 15.2 nM (0.24 – 4.53 ng/mL). Activity of artemether was similar against chloroquine-resistant (mean IC_{50} 2.8 nM i.e., 0.83ng/mL) and chloroquine-sensitive isolates (mean IC_{50}, 3.46 nM i.e., 1.03 ng/mL). A positive correlation was observed in the activity between artemether and mefloquine ($r = 0.6$, $p < .001$), artemether and quinine ($r = 0.291$, $p < 0.05$), and artemether and halofantrine ($r = 0.273$, $p < 0.05$) suggesting cross resistance between the drugs against the isolates from East African region (Table 14).

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Table 13: Activity in vitro of artemether against chloroquine sensitive and chloroquine resistant *P. falciparum* clinical isolates from Senegal in 1995 – 1996.

Table 14: Correlation between *in vitro* responses to artemether and other standard anti-malarials against *P. falciparum* clinical isolates from Senegal in 1995 – 1996

Randrianarivelojosia *et al.*,[15] measured the activity of artemether against 51 *P. falciparum* clinical isolates in Madagascar from 1998 – 1999 by incorporation of ^{3}H-hypoxanthine.. The IC_{50} values for artemether ranged between 0.23 nM and 17.5 nM (0.07 – 5.22 ng/mL). Among the isolates tested, 11% (5/46) had a high chloroquine IC_{50} value (IC_{50} > 100 nM). All isolates had low mefloquine IC_{50} values (range, 2.21 – 43.1 nM). A positive correlation was observed between the activity of artemether and chloroquine ($r = 0.51$ $p < 0.002$) but there was no observed correlation between artemether and mefloquine ($r = 0.10$ $p > 0.05$) in the Madagascar region.

Basco *et al*.,[16] measured the activity of artemether and artemisinin derivatives against 11 *P. falciparum* clinical isolates from Cambodia in 1992 by incorporation of ^{3}H-hypoxanthine method. The results in Table 15 show that the artemether IC_{50} values ranged between 3.49 nM and 10.2 nM (1.04 – 3.04 ng/mL). Artemisinin IC_{50} (range, 6.12 -19.0 nM i.e., 1.73 – 5.22 ng/mL) was 2-fold less than artemether. Among the isolates tested, all were stated as resistant to chloroquine with IC_{50} > 100 nM (range, 417 – 1260 nM), cycloguanil (range, 1120 – 25,000 nM), quinine (range, 369 – 911 nM) and pyrimethamine (range, 6720 – 26400 nM) and sensitive to halofantrine (range, 0.91 – 3.95 nM) as well as mefloquine (range, 4.62 – 28.0 nM).

Table 15: Activity in vitro of artemether and other standard antimalarial agents against *P. falciparum* clinical isolates from Cambodia in 1992

Pradines *et al.*,[17] measured the *in vitro* activity of artemether by incorporation of ^{3}H-hypoxanthine method against 63 *P. falciparum* clinical isolates from Gabon in 1997. The results in Table 16 show that artemether IC_{50} values ranged between 0.8 nM and 34.8 nM (0.23 - 10.38 ng/mL). Of the isolates tested, 90% were classified as resistant to chloroquine with IC_{50} values greater than 100 nM, 32% showed a decrease in sensitivity to quinine (mean 275 nM), and increased sensitivity to amodiaquine (mean, 10.3 nM) and halofantrine (mean, 1.5 nM). A positive correlation was observed between artemether-amodiaquine ($r = 0.68$, $p < 0.001$), artemether-chloroquine ($r = 0.60$, $p < 0.001$), artemether-quinine ($r = 0.56$, $p < 0.001$), and artemether-halofantrine ($r = 0.44$, $p < 0.001$), suggesting cross-resistance between these drugs (Table 17).

Table 16. Activity in vitro of artemether and standard anti-malarial agents against *P. falciparum* clinical isolates from Gabon in 1997

Table 17. Correlation in-vitro responses of 62 Gabonese isolates of P. falciparum to artemether

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Legrand *et al.*,[18] measured the *in vitro* activity of artemether, by incorporation of ^{3}H-hypoxanthine, against 634 *P. falciparum* clinical isolates from French Guiana from 1997 – 2005. The majority of the isolates had mean artemether IC_{50} values range between 2.2 – 3.9 nM (0.66 – 1.16 ng/mL). In 2002 and 2005, there was a trend towards a decrease in the *in vitro* sensitivity to artemether; 9 of the isolates in 2002 and 1 isolate in 2005 had an IC_{50} greater than 30 nM (8.9 ng/mL) (Figure 10). Molecular typing indicated that these isolates had a *PfATPase*6–S769N mutant allele (Table 18). Re-culture of the stored isolates with the mutant allele *PFATPase* 6-S769N in the absence of artemether for 3-weeks showed a decrease in IC_{50} value (1.42 ng/mL), suggesting a poor fitness of the mutant allele. All of the isolates showed high chloroquine IC_{50} (mean ≥ 400 nM) between 1994 and 1999; from 2000 there was a trend towards an increase in sensitivity to chloroquine (mean IC_{50}, 371 nM) and in 2005 the mean IC_{50} value for chloroquine was 96 nM. Most of the isolates showed low IC_{50} values to amodiaquine, quinine, halofantrine and atovaquone, however, proguanil/cycloguanil IC_{50} values were high from 1998 – 2005. There was no correlation noted between artemether and any of the standard anti-malarial drugs.

Figure 10: Activity *in vitro* of artemether against *P. falciparum* clinical isolates from French Guiana in 1997 to 2005

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Table 18: Susceptibility test and genotype of the artemether resistance isolate at day 0 and 21

Brockman *et al.,*[19] measured the *in vitro* activity of artemether and other artemisinin derivatives against *P. falciparum* clinical isolates from patients classified as recrudescent infection or a primary infection in Thailand. Recrudescence was defined as presenting with an infection of *P. falciparum* within 42 days of treatment with a combination of mefloquine and artesunate (MAS) in Thailand from 1995 - 1999. Activity was measured by the incorporation of ^{3}H-hypoxanthine. The results in Table 19 show that the artemether IC_{50} values ranged between 0.39 ng/mL and 17.4 ng/mL for the 26 primary infection clinical isolates and 1.1 ng/mL and 13.4 ng/mL for the 22 recrudescent clinical isolates. DHA was 2-fold more active than artemether against both primary infection (range, 0.15 – 6.6 ng/mL) and recrudescent infection (0.24 – 8.1 ng/mL) isolates. A positive correlation was observed between artemether and the other artemisinin derivatives. Artesunate, an artemisinin derivative, also showed a positive correlation between artesunate-mefloquine ($r = 0.45$, $p < 0.001$), artesunate-halofantrine ($r = 0.51$, $p < 0.001$), artesunate-quinine ($r = 0.43$, $p < 0.001$) and a negative correlation between artesunate-chloroquine ($r = -0.07$, $p < 0.04$) (Table 20).

Table 19. Activity in vitro of artemether and other standard anti-malarial agents against Thailand clinical isolates of *P. falciparum* from primary or recrudescent infections

Table 20. Correlation between response *in vitro* of Thailand clinical isolates of P. falciparum to artemisinin derivatives and lumefantrine and standard antimalarial drugs

Pradines *et al.,*[20] measured the *in vitro* activity of artemether, by incorporation of ^{3}H-hypoxanthine, against 158 *P. falciparum* clinical isolates from Senegal in 1996. The results in Table 21 show that the artemether IC_{50} values ranged between 3.6 nM and 6.8 nM (1.07 and 2.02 ng/mL). Of the isolates tested 50% (78/158) were classified as resistant to chloroquine with IC_{50} values > 100 nM. Isolates classified as chloroquine resistant were similar in sensitivity to artemether (mean IC_{50}, 5.2 nM i.e., 1.55 ng/mL) than isolates classified as chloroquine sensitive (mean IC_{50}, 5.3 nM i.e., 1.58 ng/mL). There was a positive correlation between lumefantrine and artemether IC_{50} values ($r = 0.4$, $p < 0.001$).

Table 21. Activity *in vitro* to lumefantrine and artemether and other standard anti-malarial agents against *P. falciparum* clinical isolates from Senegal in 1996

Basco *et al.,*[11] measured the *in vitro* activity of artemether by incorporation of ^{3}H-hypoxanthine against 36 *P. falciparum* clinical isolates from Africa in 1992. Of the isolates tested, 60% (21/36) were classified as chloroquine resistant with IC_{50} values >100 nM. Among the chloroquine resistant strains, clinical isolates were more sensitive to artemether (mean IC_{50}, 3.71 nM) than the chloroquine sensitive clinical isolates (mean IC_{50}, 5.14 nM), though not statistically different. Artemether showed similar activity when compared to other artemisinin derivatives, arteether and artelinate (Table 22). The results in Table 23 show that there was a positive correlation observed between artemisinin-mefloquine

($r = 0.42$, $p = 0.02$) and artemisinin-halofantrine ($r = 0.57$, $p < 0.001$), however, a negative correlation was observed between artemisinin-chloroquine ($r = -0.40$, $p = 0.018$).

Table 22: Activity in vitro to artemether and standard anti-malarial agents against P. falciparum clinical isolates

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Table 23. Correlation of in vitro responses to artemisinin and standard anti-malarial agents against *P. falciparum* clinical isolates

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The applicant provided studies by Wernsdorfer *et al.*,[21-25] that measured the *in vitro* activity of artemisinin, by microscopic method, against *P. falciparum* clinical isolates from Southern China, Thailand, and East Africa. No data were submitted to demonstrate the *in vitro* activity of artemether. Artemisinin was used as an indicator for artemether activity against clinical isolates. The authors have stated that artemether was not tested because of its instability on predosed microtitre plates. Other anti-malarial drugs were used as comparators.

In Southern China, all of the isolates showed a very low sensitivity to chloroquine and low *in vitro* response to mefloquine and quinine; similarly there was a decrease in sensitivity artemisinin with a mean EC_{50} of 652.5 nM (184.2 ng/mL) (Table 24).

In East Africa, the *in vitro* activity of artemisinin against 61 *P. falciparum* clinical isolates was measured in 1992 and 42 clinical isolates in 1993. A majority of the isolates were chloroquine resistant and had intermediate *in vitro* sensitivity to mefloquine and quinine. There appeared to be no change in the sensitivity to artemisinin since the mean EC_{50} value ranged between narrow limits of 21.06 nM and 27.37 nM (5.94 – 7.72 ng/mL) in 1992 and 1993 respectively (Table 24).

The *in vitro* activity of artemisinin against 132 *P. falciparum* clinical isolates in Thailand was measured over the period of 1994 – 1996. Most of the isolates had high chloroquine EC_{50} values and low mefloquine and quinine EC_{50} values; artemisinin mean EC_{50} value decreased from 35.85 nM (10.12 ng/mL) in 1994 to 25.91 nM (7.31 ng/mL) in 1996 suggesting there was a slight increase in sensitivity to artemisinin in Thailand (Table 24).

Table 24. Activity *in vitro* to artemether against *P. falciparum* clinical isolates from Southern China, East Africa and Thailand

3.2.2.2 *Lumefantrine*

Growth inhibition of *P. falciparum* clinical isolates from East Africa was tested by light microscopy method against the enantiomers of lumefantrine and lumefantrine racemate *in vitro*[27]. The mean EC_{50} values for lumefantrine (+), lumefantrine (-), and lumefantrine racemate were 9.8 nM (5.18 ng/mL), 7.71 nM (4.08 ng/mL), and 8.95 nM (4.73 ng/mL), respectively. The mean EC_{90} values for lumefantrine (+), lumefantrine (-), and lumefantrine racemate were 37.97 nM (20.08 ng/mL), 38.54 nM (20.38 ng/mL), and 39.48 nM (20.88 ng/mL), respectively thereby suggesting similar activity of the stereo-enantiomers of lumefantrine.

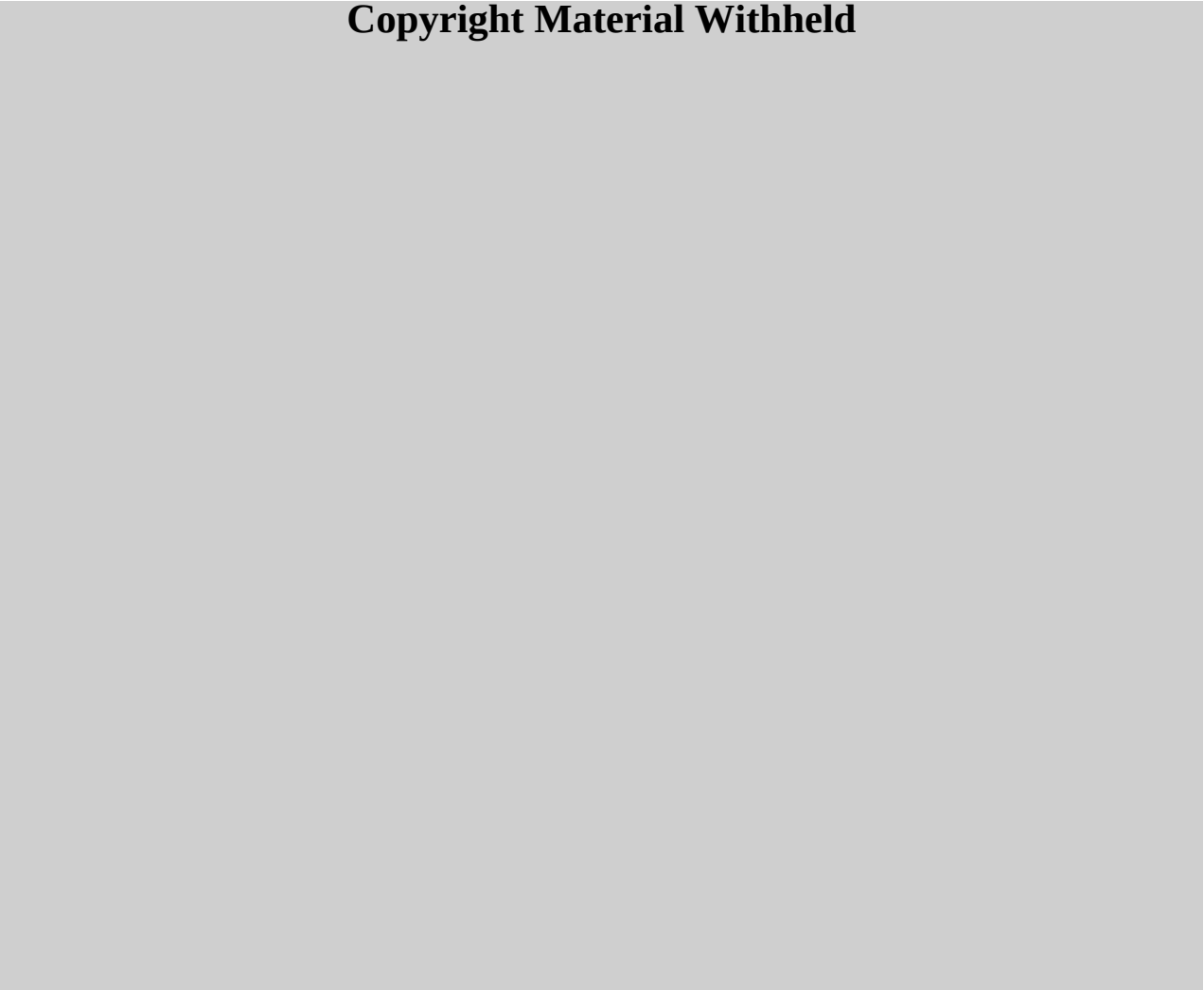
The applicant provided studies by Wernsdorfer *et al.*,[21-25] that measured the *in vitro* activity, by microscopic method, of lumefantrine against *P. falciparum* clinical isolates from Southern China, Thailand, and East Africa.

In Southern China, the *in vitro* activity of lumefantrine against 51 *P. falciparum* clinical isolates was measured in 1992. All of the isolates showed a very low sensitivity to chloroquine and to mefloquine and quinine; however the isolates showed a high sensitivity to lumefantrine with a mean EC_{50} of 24.0 nM (12.69 ng/mL) (Table 25).

In East Africa, the *in vitro* activity of lumefantrine against 64 *P. falciparum* clinical isolates was measured in 1992 and 141 isolates in 1993. A majority of the isolates were stated to be chloroquine resistant and had intermediate *in vitro* activity to mefloquine and quinine. The lumefantrine EC_{50} values were low [5.93 nM and 9. 84 nM (3.13 – 5.2 ng/mL) in 1992 and 1993, respectively] (Table 25).

The *in vitro* activity of lumefantrine against 128 *P. falciparum* clinical isolates in Thailand was measured over the period of 1994 – 1996. Most of the isolates were stated to be chloroquine resistant and also had high mefloquine and quinine EC_{50} values. The mean EC_{50} value for lumefantrine increased about 3-fold from 7.04 nM (3.72 ng/mL) in 1994 to 25.91 nM (13.7 ng/mL) in 1996 suggesting a slight decrease in sensitivity to lumefantrine in Thailand between 1994 and 1996 (Table 25).

Table 25: Activity *in vitro* to lumefantrine against *P. falciparum* clinical isolates from Southern China, East Africa and Thailand



Legrand *et al.,*[18] measured the *in vitro* activity of lumefantrine, by incorporation of ^{3}H-hypoxanthine, against 36 *P. falciparum* clinical isolates from French Guiana in 2005. The results show the mean lumefantrine IC_{50} was 156.8 nM (82.9 ng/mL). A positive correlation was observed between lumefantrine and halofantrine ($r = 0.78$; $p < 0.0001$) but no correlation was observed with mefloquine.

Brockman *et al.,*[19] measured the *in vitro* activity of lumefantrine against 32 *P. falciparum* clinical isolates from patients classified as recrudescent infection or a primary infection. Recrudescence was defined as presenting with an infection of *P. falciparum* within 42 days of treatment with a combination of mefloquine and artesunate (MAS) in Thailand from 1995 - 1999. Cultures were examined using the ^{3}H-hypoxanthine method. The results in Table 26 show that the lumefantrine IC_{50} value for the 20 primary infection clinical isolates, range between 2.0 ng/mL and 75.6 ng/mL, compared to the 12 recrudescent isolates with the IC_{50} range between 20.2 ng/mL and 87.1 ng/mL. No positive correlation was observed between lumefantrine and other standard anti-malarial agents.

Pilz *et al.,*[28] measured the *in vitro* activity of lumefantrine, by light microscopy, against 41 *P. falciparum* clinical isolates from Mae Sot, Thailand in 2002. The results in Table 27 show that the lumefantrine EC_{50} value ranged between 15.13 nM and 41.27 nM (8.0 – 21.8 ng/mL) for the period 1998 – 2002 thereby suggesting a decrease in sensitivity to lumefantrine.

Pradines *et al.,*[20] measured the *in vitro* activity of the enantiomers and racemic mixture of lumefantrine, by incorporation of ^{3}H-hypoxanthine, against 158 *P. falciparum* clinical isolates from Senegal in 1996. The results in Table 28 show that lumefantrine IC_{50} values ranged between 12.5 nM and 240 nM (6.61 – 126.9 ng/mL). Of the isolates tested 50% (78/158) were classified as resistant to chloroquine with IC_{50} values > 100 nM. Isolates classified as chloroquine resistant were more sensitive to lumefantrine [mean IC_{50}, 47.9 nM (25.3 ng/mL)] than isolates classified as chloroquine sensitive [mean IC_{50}, 63.4 nM (33.5 ng/mL)]. Table 29 shows a positive correlation between lumefantrine-artemether ($r = 0.4$, $p < 0.001$), lumefantrine-quinine ($r = 0.37$, $p < 0.001$), lumefantrine-pyronaridine ($r = 0.32$, $p < 0.001$), and lumefantrine-amodiaquine ($r = 0.22$, $p < 0.001$). Lumefantrine IC_{50} values did not correlate significantly with chloroquine and pyrimethamine IC_{50} values.

Table 28: Activity *in vitro* to lumefantrine and artemether and other standard anti-malarial agents against *P. falciparum* clinical isolates from Senegal in 1996

Table 29: Correlation of in vitro responses of Senegal clinical isolates of *P. falciparum* to lumefantrine and other standard anti-malarial drugs

3.3 ACTIVITY *IN VIVO* (ANIMAL STUDIES)

3.3.1 Activity in mice infected with *Plasmodium berghei*

3.3.1.1 *Artemether*

Shmuklarsky *et al.*,[10] measured the activity of β-artemether in young CD-1 Swiss mice injected subcutaneously with 6 x 10^5 erythrocytes parasitized with the *P. berghei* KBG 173 strain. Groups of 5 or 10 mice were subcutaneously administered a starting drug dose of 640 mg/kg followed by a four fold lower decrease, 72 hour after inoculation. Once a drug showed activity on initial screening, it was further tested at additional dose levels obtained by 2-fold dilution. The tests were performed at lower doses until the lower limit of activity was reached. Young untreated control mice injected with a standard inoculum of *P. berghei* served as controls. All mice were observed until death; surviving animals were followed up to 60 days. Cultures were examined using the ^{3}H-hypoxanthine method, however, the results of such testing were not shown. It was stated that the activity of β-artemether and β-arteether was similar. The 50% curative dose (CD_{50}) for β-artemether was stated as 55 mg/kg (range,

32 – 78 mg/kg). No data were shown on the number of animals that survived or clearance of parasitemia. It is unclear what the CD_{50} value represents.

3.3.1.2 *Lumefantrine*

The activity of the stereo-enantiomers of lumefantrine was measured in hybrid Swiss albino KM mice infected with 1x 10^7 parasitized erythrocytes of *Plasmodium berghei* (Keyberg 173 N strain)[29]. A total of 144 animals, 12 groups of 12 mice, were treated on days 0, 1, 2, and 3 with either lumefantrine (+) or lumefantrine (–) at different doses (0, 2, 4, 8, 16 or 32 mg/kg) via the intra-gastric route. Untreated mice served as controls. All mice were observed until death and surviving animals until day 30. Parasitemia was determined on days 4 and 30, if the mice survived, by examining blood smears, stained using 1.5% Giemsa stain solution. In the untreated mice, all animals died with a mean time to death of $\leq 8.58 \pm 1.44$ days (Table 30). Both of the stereo-enantiomers were effective in improving 30 day survival and day 4 parasitemia, at a dose of ≥ 8 mg/kg. Parasitemia before and after day-4 dosing was not shown. Based on survival the mean effective dose (EC_{50} and EC_{90}) values for (+) lumefantrine (6.97 mg/kg and 18.65 mg/kg, respectively) and (–) lumefantrine (4.7 mg/kg and 16.30 mg/kg) were similar.

Table 30: Activity of the stereo-enantiomers of lumefantrine in the *Plasmodium berghei* infected mice

3.3.1.3 *Combination of artemether and lumefantrine*

The activity of lumefantrine and artemether alone or in combination was examined in male TFW mice inoculated intravenously with 10^7 parasitized erythrocytes of *P. berghei* Keyberg 173 N strain[30]. At the time of infection, treatment was initiated for 4 days with increasing oral doses of artemether (range 0.25 – 4.0 mg/kg), lumefantrine (range 0.1875 – 2.0 mg/kg), a combination of artemether and lumefantrine or a vehicle. Blood smears were stained with Giemsa to calculate the relative percentage inhibition of parasitemia and the ED_{50} and ED_{90} values. A cure was defined as the ability of the drug

given at highest ED_{90} value to clear infection by day 30. The results in Table 31 show that the ED_{50} and ED_{90} values for artemether were 2.10 mg/kg and 7.50 mg/kg, respectively. The ED_{50} and ED_{90} values for lumefantrine were 1.27 mg/kg and 2.99 mg/kg, respectively. A combination of artemether and lumefantrine was more effective than either artemether or lumefantrine alone. No antagonism was observed when the ED_{90} values of artemether and lumefantrine were plotted as an isobologram (Figure 11). The time required for reducing the parasitemia by 50% was 2 times faster in mice treated with artemether (mean, 23 hours) compared to that of lumefantrine (mean, 54 hours) (Table 32). Treatment with lumefantrine resulted in clearance of parasitemia; whereas treatment with artemether often resulted in recrudescence of infection. The combination of artemether and lumefantrine in a ratio of 1:0.375 resulted in a faster reduction in parasitemia similar to that of artemether alone and survival in all animals similar to that of lumefantrine alone.

Table 31: *In vivo* activity of lumefantrine alone, artemether alone or combination of artemether and lumefantrine in mice infected with *P. berghei*

Figure 11: Isobologram comparing the ED_{90} of artemether versus lumefantrine

Table 32: Reduction of parasitemia in mice infected with *P. berghei* when treated with lumefantrine alone, artemether alone or combination of artemether and lumefantrine

3.3.2 *Activity in monkeys infected with Plasmodium* sp.

3.3.2.1 *Artemether*

Shmuklarsky *et al.*,[10] measured the activity of β-artemether in 58 Panamanian owl monkeys (*Aotus lemurinus lemurinus*) infected intravenously with 5 x 10^5 trophozoites in 1 ml (obtained from infected untreated monkeys) of the *P. falciparum* Vietnam Smith/RE strain.. On day 5 post-inoculation, drugs were administered intramuscularly in 3 doses at 12 hour intervals. Thick blood films were performed until blood films were parasite negative for at least 7 days. Untreated and treated monkeys whose parasite counts continued to increase for several days after treatment (the exact day not specified) or whose parasitemia recrudesced were treated with mefloquine and were included by the authors in analysis. A cured infection was defined as no evidence of parasitemia for 100 days. Parasitic clearance was defined as first 3 consecutive days in which the blood films were negative for parasites. The day of recrudescence was determined as the first 3 consecutive days of positive thick blood films after a period of clearance. Suppression was defined as transient decrease in the parasite count post-treatment without clearance. Results in Table 33 show that artemether was effective in reducing parasitemia at a dose of ≥ 12 mg/kg in all the animals (n=4 to 8). Recrudescence was observed at ≥ 24 mg/kg. Results of the untreated group were not shown. The 50% curative dose (ED_{50}) for β-artemether was 7.1 mg/kg (95 confidence intervals, 3.7 – 13.5 mg/kg) and similar to arteether. These results include animals administered with mefloquine, and therefore, should be interpreted with caution.

Table 33: *In vivo* activity of β-artemether and β-arteether in Panamanian owl monkeys infected with Vietnam Smith/RE *P. falciparum* strain

3.3.2.2. *Combination of artemether and lumefantrine*

The activity of artemether and lumefantrine alone or in combination was measured in rhesus monkeys (*Macaca mulatta)* infected with 5x 10^8 parasitized erythrocytes of *Plasmodium knowlesi*[31]. A total of 18 monkeys were placed into 6 groups of 3 monkeys each. Animals were treated with 4 doses of the drug by the intra-gastric route. Treatment was initiated after parasitemia of 3 – 5% was established in the animals. The treatment consisted of a 3-day schedule (0, 12, 24 and 48 hours) with artemether alone, lumefantrine alone, combination of artemether and lumefantrine in a 1:4 and 1:6 ratios. Few animals were also treated with a 4-day schedule (0, 24, 48 and 72 hours) of artemether and lumefantrine in the ratio of 1:4. Blood smears were performed and stained with 1.5% Giemsa to examine for parasites at 12-hourly intervals until the third negative blood smear after treatment. Blood smears were examined thereafter every day until day-15 and every 2 days between days 15 and 105. The results in Table 34 show that treatment with artemether (2 mg/kg) alone was effective in reducing parasitemia but failed to clear the parasitemia. None of the animals in the artemether alone treatment group were completely cured. Treatment with lumefantrine alone at a lower dose (12 mg/kg) resulted in a slower reduction of parasitemia and only 1 animal developed recrudescence. At a higher dose (16 mg/kg), treatment with lumefantrine alone was effective in rapid reduction of parasitemia; 2 animals developed recrudescence. The 3-day treatment regimen of the combination of artemether and lumefantrine (1:4 ratio) had the lowest mean time to parasite clearance (48.0 ± 0.0 hours) compared to other groups; none of the animals developed recrudescence. Though the treatment regimen of 3-day with a combination of artemether and lumefantrine in a 1:6 and 1:4 ratio were similar in improving survival and time to 90% reduction of parasitemia , the mean time to parasite clearance, survival and

prevention of recrudescence were higher in animals treated with the 1:4 combination. The 4-day treatment regimen of 1:4 ratio artemether and lumefantrine was less effective compared to the other combinations in parasite clearance and the overall survival. However, the numbers of animals per treatment group were very small.

Table 34: Activity of artemether and lumefantrine alone or in combination in rhesus monkeys infected with *P. knowlesi*

3.4. DRUG RESISTANCE

3.4.1. ACTIVITY *IN VITRO*

The development of drug resistance to lumefantrine and artemether alone or in combination were examined *in vitro* against the chloroquine-resistant *P. falciparum* K1 strain[32]. Cultures of parasitized erythrocytes were exposed to concentrations below the IC_{50} concentrations of each drug. Cultures of parasitized erythrocytes in drug-free medium served as controls. The medium was changed daily and cells diluted to 1% parasitemia every 2 to 3 days. The number of passages was not specified. It was stated that parasitemia was determined as a percentage of the multiplication rates in the presence and absence of drug. Parasitemia that were below 40% were grown in drug-free medium until normal parasite growth **5**was obtained. Here again, the number of passages was not specified. The results showed no decrease in sensitivity to lumefantrine, artemether, or the combination of the *P. falciparum* K1 strain.

3.4.2. ACTIVITY *IN VIVO*

Ward *et al.,*[32] determined drug resistance to lumefantrine and artemether alone or in combination in male TFW mice inoculated intravenously with 10^7 parasites of the N and NS strain of *P. berghei.* The experiments with N strain of *P. berghei* were discontinued because of erratic and unreliable results. Mice were treated with daily doses of drug for 4 days, at the time of infection with *P. berghei* NS strain. The *in vitro* IC_{50} and IC_{90} values were determined on the day following the last dose. Drug induction studies were conducted in naïve mice using inoculums corresponding to a 2% reduction in parasitemia by day-7 in the donor mice. Mice at the time of induction with the parasitized erythrocytes

from a donor mice were administered a single dose of the drug. The control group did not receive drug treatment. Blood samples were taken from the mice daily, percentage parasitemia and *in vitro* drug sensitivity between the passage strain and parent strain were examined using the 4-day suppressive test method of inhibition of parasite. However, the results of *in vitro* sensitivity testing were not included. The results in Figure 12A show that in drug induction studies using lumefantrine alone, after 7 passages of the NS strain, the effective dose decreased by 9-fold (0.90 mg/kg). The strain was passaged up to 18 times and the activity of lumefantrine against the 18th passaged strain, labeled as NSBF2.18, was less active than against the parent NS strain (Figure 12B). However, the *in vivo* sensitivity of the strain NSBF2.18 was shown to be similar to the parent strain (NS) in mice treated with a combination of artemether and lumefantrine at a ratio of 1:6 (Figure 12C). Drug induction studies using artemether alone showed unstable activity (Figure 13). The activity of artemether against the 29th passaged strain on day 220 (2.01 mg/kg) was more active than against the parent NS strain (3.24 mg/kg) (Figure 13). Drug induction studies using a combination of artemether and lumefantrine in the ratio of 1:6 also showed unstable drug activity similar to that of artemether (Figure 14). The activity of combination of artemether and lumefantrine (0.004 mg/kg : 0.02 mg/kg; ratio 1:6) against the 18th passaged strain on day 150 was similar to the activity against the parent NS strain (0.009 mg/kg : 0.06 mg/kg).

Figure 12. Comparison of the sensitivity to lumefantrine of *P. berghei* NS parent line and the developing strain NSBF2.18. (A) period of time to development of resistance, (B) in *vitro* sensitivity of strain to lumefantrine, and (C) in *vitro* sensitivity of strain to drug combination of artemether and lumefantrine in a 1:6 ratio.

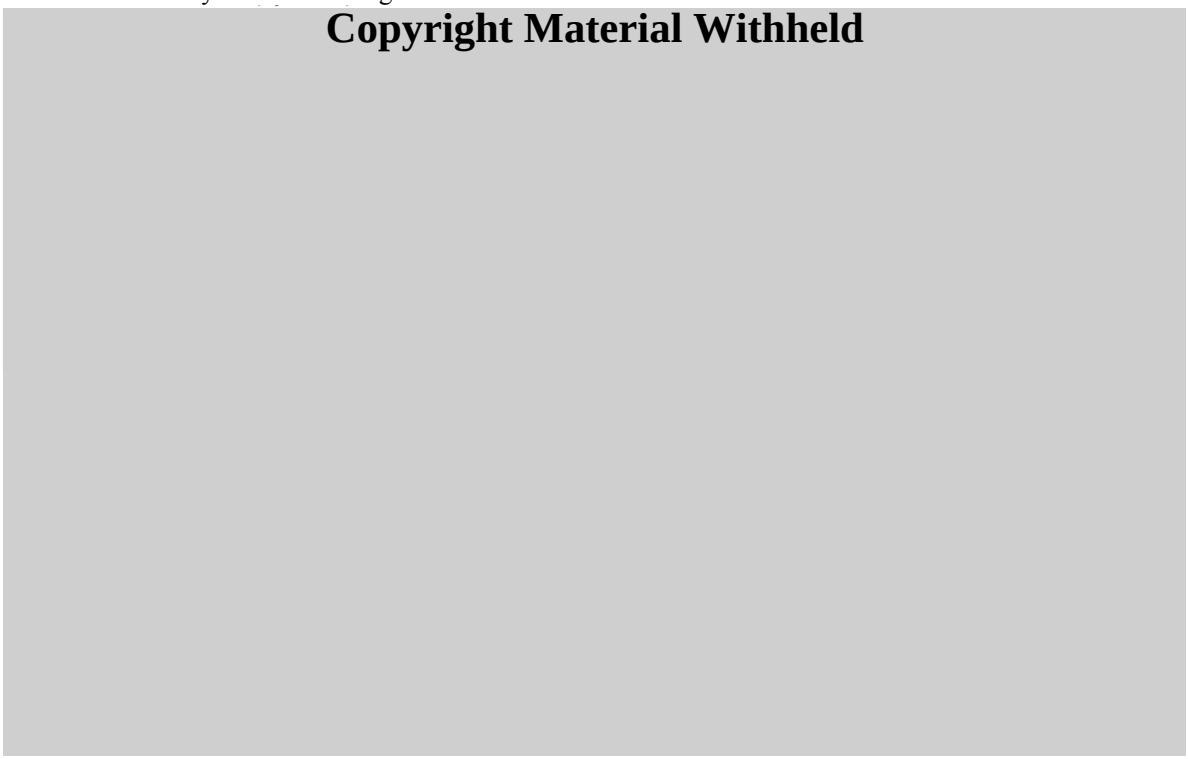


Figure 13: Comparison of the sensitivity to artemether of *P. berghei* NS parent line and the developing strain NSART29. (A) time to development of resistance (B) in *vitro* sensitivity of resistant strain and parent strain to artemether

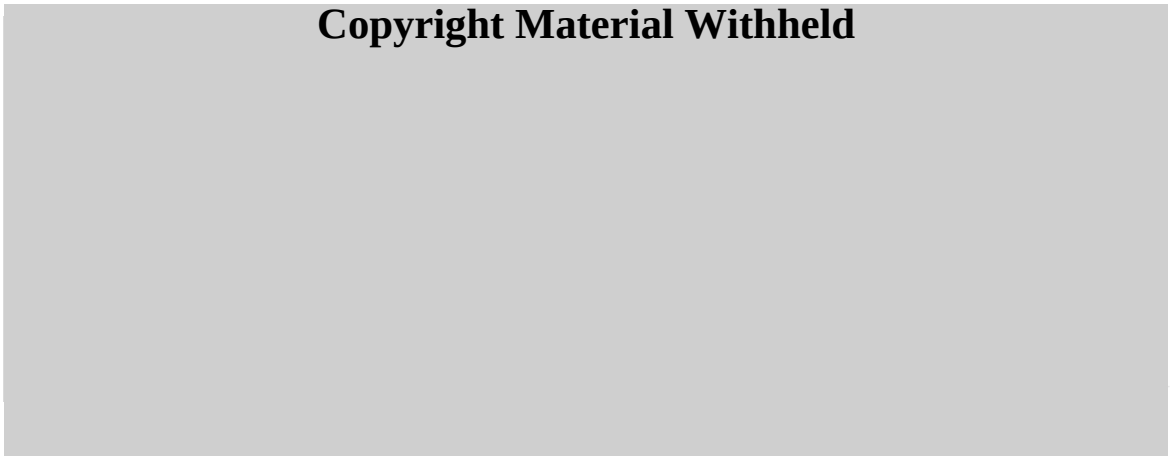
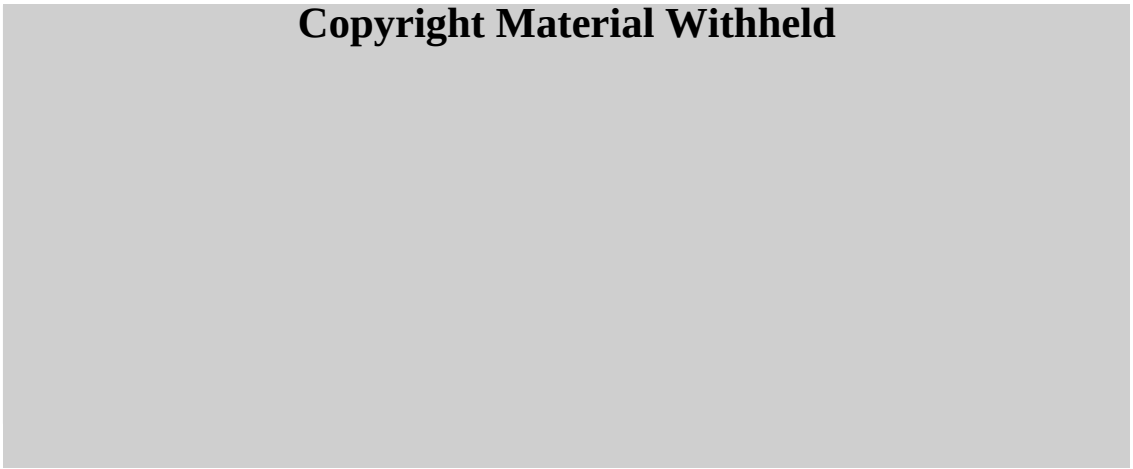


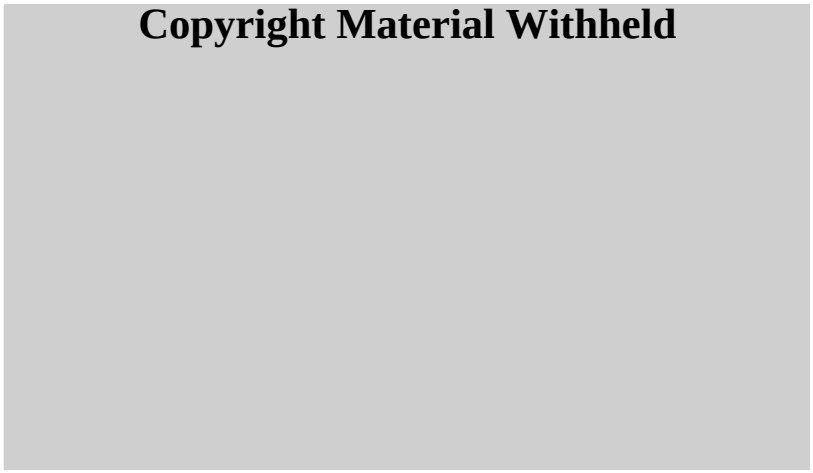
Figure 14: Comparison of the sensitivity to combination of artemether and lumefantrine in a 1:6 ratio of *P. berghei* NS parent line and the developing strain NSCombi.18. (A) time to development of resistance, and (B) in *vitro* sensitivity of resistant strain and parent strain to drug combination of artemether and lumefantrine.



In another study[33], the development of resistance to artemether, lumefantrine and the combination of lumefantrine and artemether was examined against established infections of *P. berghei* Keyberg 173 N strain and passaged *in vivo* using male Swiss mice under the influence of progressively increasing drug doses. Mice were intra-peritoneally injected with 10^7 parasitized erythrocytes. On day 3 when parasitemia was established, mice were treated with daily doses for 3 days. On day 7, blood smears were prepared and stained with Giemsa to assess the parasite count. Blood from an infected donor mouse with the highest parasite count was prepared as above and used for passage into a new group of mice. The inoculum concentration corresponded to doses which permitted an increase of parasitemia beyond the threshold of 2% by day 7. The ED_{50} and ED_{90} were assessed at intervals of 5 passages and

results expressed as the inhibition of schizonticidal activity of the drug using the 4-day suppressive test method. In this test, mice were infected intra-peritoneally with 10^7 parasitized erythrocytes and given a single dose of the drug at the time of passage. The results show a 10-fold increase in the artemether ED_{90} after 50 passages as compared to first passage (Figure 15). The strain decreased in sensitivity to artemether (55 mg/kg) after the 60^{th} to 80^{th} passage. The lumefantrine ED_{90} increased about 400-fold (from 2.5 mg/kg to 1000 mg/kg) after the 30^{th} passage, and stayed uniformly high at this dose up to the 80^{th} passage. After the first passage for the 2:0.75 combination of artemether and lumefantrine the ED_{90} was 2.9 mg/kg. There was a 42-fold increase in the ED_{90} for the combination of artemether and lumefantrine (138 mg/kg) after the 45^{th} passage and plateau to an ED_{90} of 92 mg/kg after the 65^{th} passage.

Figure 15: ED_{90} to artemether, lumefantrine and a 2:0.075 combination of artemether and lumefantrine in male Swiss mice infected *P. berghei* Keyberg 173 N strain



4. CLINICAL MICROBIOLOGY

4.1 Description of the clinical study/studies

Of the 24 clinical studies submitted by the applicant, the datasets were available for 8 studies (025, 026, 028, 023, 2401, 2403, 2303 and ABM02) and are described in detail. The additional 16 studies (2412, 1003, 1004, 1005, 1007, 1008, 1010, 1011, 1014, 030, BD01, BR01, IC04, ABM01 and 1009) were considered supportive and are summarized briefly.

4.1.1 Study AB/M02

Study AB/MO2 was a randomized, active controlled, 3-arm, double blind, single center (Institution of Microbiology and Epidemiology at the Academy of Military Sciences, Beijing, China) trial that enrolled 157 patients, between the ages of 13 and 60 years, with mild to moderate symptomatic falciparum malaria and a parasite count of 1,000-100,000/µl. Patients infected with any *Plasmodium*

species other than *P. falciparum* were excluded. The objectives of the study were to compare the efficacy and tolerability of 4 doses at 0, 8, 24, and 48 hours of Coartem (120 mg lumefantrine / 20 mg artemether) with each of its components, artemether (20 mg) and lumefantrine (120 mg). The primary endpoints of the study included the 28 day cure rate (clinical and parasitological), the time to parasite clearance (PCT) and the time to fever clearance (FCT). Secondary endpoints included examining anti-gametocyte activity and determining the parasite reduction at 24 hours. Anti-gametocyte activity was defined as the clearance of existing gametocytes without the need for additional anti-malarials.

Parasitological Measurements
Parasite counts were taken at baseline and every 6 hours thereafter for the first 72 hours. Some patients also had 6 or 12 hour counts on day 4. Thereafter, parasite counts were taken daily until day 28. Patients remained at the test facility until day 29. Any reemergence of infection was assumed to be recrudescence. Parasite counts were performed on Giemsa stained thick films wherein the count was stopped when either the number of parasites or the number of WBCs reached 500. The protocol specified that parasite density be calculated using the patient's actual WBC count. No thin films were examined and it appears that only 1 slide was examined per time point (per patient) by one microscopist.

For quality control, thick films were reexamined. Forty baseline parasite slides, 40 slides taken during the trial to confirm parasite clearance and 55 slides taken at follow-up, irrespective of the treatment arm, were evaluated at the (b) (4) in Basel, Switzerland. Upon comparing the results between the initial analysis and the quality control analysis, it was found that 6 (15%) of the 40 baseline slides differed by 2- to 10-fold. Four (10%) of the slides used to confirm parasite clearance showed discordant results (note: these slides were graded as positive or negative). Of the 55 slides reexamined at follow-up, 7 (13%) showed discordant results; this includes one slide from a patient in the Coartem arm which was graded as negative during the trial, though upon reexamination was found to be positive. The center performing the quality control does, however, note that when parasite counts were very low, the entire slide was examined and was not stopped at the 500 parasites or WBC limit described above. This may have greatly increased the sensitivity of the analysis and thus changed the overall results.

Parasitological Findings
Asexual parasites
The median (mean) baseline parasite counts per µL were 23,479 (19,431), 19,602 (20,386) and 26,697 (22,415) for the Coartem, artemether and lumefantrine arms, respectively. Twelve patients were included in the trial that had baseline counts which were higher than 100,000 parasites/µL of blood. The applicant stated that these patients should not have been included in the trial since the exclusion range was <1,000 or >100,000 parasites/µL of blood. However, for the purpose of this review the results are included in the analysis.

Of the 157 patients in ITT [Coartem (n=53), artemether (n=52) and lumefantrine (n=52)] population (i.e., patients receiving at least one dose), 6 patients withdrew from the trial prematurely and 6 patients in the artemether arm received rescue medication due to the diagnosis of *P. vivax* infection after the trial began. There is no evidence provided that these patients were able to clear infection with *P. vivax* since four of the patients were withdrawn at the time of *P. vivax* diagnosis and two patients were

unable to clear infection with *P. vivax* from diagnosis (day 23) until the last measurement (day 31). All 12 patients are excluded from the per protocol population (Table 35). As can be seen from the data presented in Table 35, Coartem is equivalent to artemether in parasite clearance time but is superior in its ability to prevent recrudescence. Furthermore, the data indicate that patients treated with artemether alone are at higher risk for emergence of *P. vivax*. When comparing Coartem to lumefantrine, Coartem is superior in the speed of its effect (i.e. shorter PCT). This is particularly highlighted in the observation that 26 of the 51 per protocol patients treated with lumefantrine actually had their parasite burdens increase above baseline at some point during the first 48 hours of treatment. Coartem was also superior to lumefantrine in its ability to prevent recrudescence.

Using the WHO classification of response to therapy, 24 patients were considered R-I treatment failures due to recrudescence of *P. falciparum* within the study period (Table 36). Of these 24 recrudescence cases, 20 occurred in the artemether group between days 12 and 28 and 4 in the lumefantrine group between days 25 and 28. Two patients in the lumefantrine group could be classified as R-III failures (parasite count of >25% of the baseline value after 48 hours). The applicant states that due to the slow action of lumefantrine, an argument can be made that this classification is misplaced. Of the two patients with R-III failure, one patient was later classified as cured and the other was not.

Table 35: The clearance of *P. falciparum* over time in response to Coartem or its individual constituents

Parameter		Coartem	Artemether	Lumefantrine
Number of patients**	**ITT (n=155)**	51	52	52
	PP (n=145)	50	44	51
Baseline parasitemia/µL [median (range)]	**ITT**	23479 (1038 – 398880)	19602 (1568 – 388000)	27592 (1240 – 162771)
	PP	22802 (1038 – 398880)	19286 (1568 – 388000)	27300 (1240 – 162771)
Percentage Parasite Reduction at 24 hours* [median (range)]	**ITT*****	99.3 (29.3 – 100)	99.9 (0 – 100)	78.2 (-4016 – 100)
	PP	99.3 (29.3 – 100)	99.9 (0 – 100)	77.2 (-4016 – 100)
% patients in which parasites are cleared by 24 hours	**ITT**	31% **	46%	3.8%
	PP	32%	45.5%	3.9%
Percentage Parasite reduction at 48 hours [median (range)]	**ITT**	100 98.5 - 100	100 99.4 - 100	99.49 -207 - 100 ****
	PP	100 98.5 - 100	100 99.4 - 100	99.51 -207 - 100
Percentage Parasite reduction at 72 hours [median (range)]	**ITT**	100 100 - 100	100 100 - 100	100 91.7 - 100
	PP	100 100 - 100	100 100 - 100	100 91.7 - 100
% Cure rate at 7 days	**ITT**	100	100	100
	PP	100	100	100
% Cure rate at 14 days	**ITT**	100	98	100
	PP	100	98	100
% Cure Rate (28 days) *	**ITT**	94.3	46.2	90.4
	PP	100	54.5	92.2
PCT*, ** [mean hours (range)]	**ITT**	33.17** 24- 54	29.65 18 - 66	54.34 24 - 90
	PP	33.24 24- 54	29.86 18 - 66	54.23 24 - 90
Recrudescence	**ITT**	0	20	4
	PP	0	19	4

* The per protocol (PP) cure rate, PCT and PC@24hr excludes 12 patients who discontinued due to reasons other than reappearance of *P. falciparum* asexual forms
** IMPORTANT: Patients 71 and 230 (both were Coartem patients) were excluded in the ITT and PP analysis as their PCT was listed as 0 in the EFF dataset. *** For 2 patients parasite count was not determined at 24 hours
**** For 1 patient parasite count was not determined at 48 hours

Table 36: Treatment failures as classified by the World Health Organization* in the PP population

Treatment Group	R-I	R-II	R-III
Coartem (n=50)	0	0	0
Artemether (n=44)	20	0	0
Lumefantrine (n=51)	4	0	2

*WHO definitions of treatment failure
R-I= clearance of asexual parasitemia within 7 days, followed by recrudescence
R-II=marked reduction of asexual parasitemia but no clearance (asexual parasite counts of <25% of baseline within 48 hours after initiation of treatment but no, or only temporary clearance of asexual parasitemia within 7 days
R-III=no marked reduction of asexual parasitemia (asexual parasite counts remain >25% of baseline at 48 hours, or actually rise above baseline levels at 48 hours without clearance of asexual parasitemia within 7 days.

Gametocytes

A gametocyte count was performed for all patients at all visits. At baseline, only 5 patients (3 patients in the artemether arm and 2 in the lumefantrine arm) had positive counts (Table 37). The gametocyte clearance time (range: 11 to >28 days) in both the artemether and lumefantrine arms was at least 10 fold higher than each patient's asexual parasite clearance time. In 9 patients who had gametocytes emerge following treatment initiation (time to emergence range: 12 hours to 12 days), gametocyte clearance time ranged between 8 and >28 days (Table 38). There were only 2 patients in the artemether arm with gametocytes detected at 12 and 24 hours after initiation of treatment.

Table 37: Patients with gametocytes present at baseline*

Treatment Group	Patient ID number	Baseline Gametocyte Count per µL	Time to Gametocyte Clearance (days)	Asexual parasite clearance time (hours)
Artemether (n=3)	112	2600000	Positive on day 28 (count:97000)	24
	208	1351000	11	24
	211	432000	19	18
Lumefantrine (n=2)	61	227000	22	54
	201	57000	Positive on day 28 (count:99000)	36

* Clearance time for those patients who did not clear the gametocytes was censored at: (day 29-initial time of gametocyte detection)

Table 38: Patients with no detectable gametocytes at baseline whose first occurrence of gametocytemia occurred after initiation of treatment*

Treatment Group	Patient ID number	Gametocyte Count/µl (Time reported)	Time to gametocyte Clearance (days)	Asexual PCT of patient (hours)
Coartem (n=2)	82	99000 (12 hours)	15	36
	237	148000 (24 hours)	11	30
Artemether (n=2)	64	87000 (18 hours)	gametocytes present on Day 28 (count=34000)	24
	102	185000 (12 days)	8	24
Lumefantrine (n=5)	68	78000 (2.3 days)	15	48
	128	370000 (30 hours)	26	60
	129	68000 (24 hours)	11	60
	148	63000 (4 days)	15	72
	222	446000 (30 hours)	21	36
* Clearance time for those patients who did not clear the gametocytes was censored at (hour 690-initial time of gametocyte detection)				

4.1.2. Study 023 (Protocol # 5669701 023)

Study 023 was a 3-arm, randomized, parallel group, comparative trial of Coartem and one of its components, benflumetol (i.e., lumefantrine tablets and capsules) in patients with *Plasmodium falciparum* infection with baseline parasitemia of >1,000 and <150,000 parasites per µL in China (Institute of Microbiology and Epidemiology at the Academy of Military Sciences, Beijing; same site as for study ABM02). Dosing of each treatment occurred as follows: 4 doses over 48 hours (hours 0, 8, 24, 48) with either Coartem (480 mg lumefantrine and 80 mg artemether; n=52) or 480 mg of lumefantrine tablets (n=51). Lumefantrine capsules (n=51) were dosed as 800 mg at hour 0 and 400 mg at 24, 48 and 72 hours. The reason for the difference in dosing schedules of each of the lumefantrine formulation is unknown. The duration of the trial was 28 days and the endpoints were PCT, parasite reduction at 24 hours, 28 day cure rate, and anti-gametocyte activity.

Parasitological Measurements

Parasite counts were performed on Giemsa stained thick films every 6 hours for the first 72 hours, parasite counts were then performed once daily until day 8 and then weekly (days 8, 15, 22 and 29). If infection reemerged, daily parasite counts were reinstated. At screening, 20 thick film fields were examined. If asexual forms were found, 200 thick film fields were examined for species other than *P. falciparum*. Counting was based on the number of parasites found per 200 leukocytes; if a low number of parasites were counted then the number of leukocytes was extended to 500. The protocol specified that parasite density was to be calculated according to the following formula: Parasites/µL= number of parasites x patient's white blood cell count (WBC) count per µL at baseline / number of leukocytes counted (200). In order for a slide to be declared negative, a total of 200 fields per slide were examined. If gametocytes were seen during examination of slides, a gametocyte count was performed until 1000 WBCs were counted. If the number of gametocytes per 1000 WBCs was less than 5, then 2000 WBCs were counted. It appears that only one count was performed per slide and quality control was not performed on slides used for parasitological counting. The applicant stated that given the low

failure rate and "excellent agreement from a previous trial in China" (study not referenced), that this peer review was not necessary.

Parasitological Findings

Asexual Parasites

The intent to treat population consisted of 153 patients, while the per protocol population consisted of 149 patients (51 in the Coartem arm, 49 in the lumefantrine tablet arm and 49 in the lumefantrine capsule arm). Patients were enrolled if their baseline parasitemia was >1,000 and <150,000 parasites per μL. It is important to note that the Coartem group had a lower mean baseline parasite count than the lumefantrine groups. While this may have introduced some bias in the parasitological response (PCT, reduction in parasitemia, and 28 day cure rates), an independent analysis performed by a statistician showed no bias as similar parasitological response was observed in patients with similar baseline counts in different treatment groups (for details see Statistician's review).

Results in Table 39 show that Coartem is more effective in reducing PCT and parasite count at 24 and 48 hours than either of the dosage forms of lumefantrine alone. This is particularly highlighted in the observation that 22 and 25 patients treated with lumefantrine capsules and tablets, respectively, had their parasite burdens increase above baseline at some point during the first 48 hours of treatment whereas only 10 patients in the Coartem arm experienced a similar increase. The effects of both dosage forms of lumefantrine appear to be equivalent with few subtle differences. Furthermore, the data indicate that patients treated with Coartem have a lower incidence of recrudescence compared to lumefantrine alone though this difference may not be statistically significant (Table 40).

Table 39: The clearance of *P. falciparum* over time in response to Coartem or lumefantrine

Parameter		Coartem	Lumefantrine capsules	Lumefantrine tablets
Number of Patients	**ITT** (n=153)	52	50	51
	PP (n=149)	51	49	49
Baseline parasitemia/µL [median (range)]	**ITT**	11778 (1288 – 95374)	23780 (1103 – 127281)	25508 (1026 – 148626)
	PP	11753 (1288 – 95374)	24185 (1103 – 127281)	27163 (1026 – 148626)
Percentage Parasite Reduction at 24 hours [median (range)]	**ITT**	99.9 (63.1 – 100)	86.7 (-1487 – 100)	78.7 (-46.9 – 100)
	PP	99.9 (63.1 – 100)	86.7 (-1487 – 100)	78.7 (-46.9 – 100)
% patients in which parasites are cleared by 24 hours	**ITT**	40.4	6	3.9
	PP	41.2	4.1	2.0
Percentage Parasite reduction at 48 hours [median (range)]	**ITT**	100 (93.04 – 100)	99.8 (-112.3 – 100)	100 (35.92 – 100)
	PP	100 93.04 – 100	99.8 -112.3 – 100	99.99 35.92 - 100
Percentage Parasite reduction at 72 hours [median (range)]	**ITT**	100 (100 – 100)	100 (66.2 – 100)	100 (99.7 – 100)
	PP	100 (100 – 100)	100 (66.2 – 100)	100 (99.7 – 100)
% Cure rate at 7 days	**ITT**	98	98	98
	PP	100	100	100
% Cure rate at 14 days	**ITT**	98	96	96
	PP	100	100	98
% Cure Rate (28 days)	**ITT**	96.2	94.0	92.1
	PP	98	95.9	91.8
PCT [mean hours (range)]	**ITT**	30.67 (11.9 – 72)	54.2 (17.8 – 107.9)	50.9 (23.9 – 84.0)
	PP	29.9 (11.9 – 66)	54.9 (24 – 107.9)	51.7 (24.0 – 84.0)
Recrudescence	**ITT**	2	3	6
	PP	1	2	4

Table 40: Treatment failures as classified by the World Health Organization*

Treatment Group	R-I	R-II	R-III
Coartem (n=52)	1	0	0
Lumefantrine capsules (n=50)	2	0	0
Lumefantrine tablets (n=51)	4	0	0

*WHO definitions of treatment failure:
R-I= clearance of asexual parasitemia within 7 days, followed by recrudescence
R-II=marked reduction of asexual parasitemia but no clearance (asexual parasite counts of <25% of baseline within 48 hours after initiation of treatment but no, or only temporary clearance of asexual parasitemia within 7 days
R-III=no marked reduction of asexual parasitemia (asexual parasite counts remain >25% of baseline at 48 hours, or actually rise above baseline levels at 48 hours without clearance of asexual parasitemia within 7 days.

***P. vivax* co-infection**

The applicant states that only 3 patients (1 from each treatment arm) had *P. vivax* co-infection at baseline and that each patient rapidly cleared *P. vivax* and there was no evidence of relapse or recrudescence of the parasite during the trial. However, information supporting *P. vivax* parasite clearance time was not available. Two other patients had emergence of *P. vivax* during the trial (Patient 28 in the Coartem arm and Patient 30 in the lumefantrine capsules arm) on days 22 and 28, respectively.

Gametocytes

A gametocyte count was performed for all patients at all visits. At baseline, 8 patients had positive counts (Table 41). The gametocyte clearance time ranged from 30 hours to >28 days and a difference between treatment groups is not apparent based on the small number of patients in each of the treatment arm. It can be noted that the Coartem arm is the only treatment group (n=4) with a gametocyte clearance time under 50 hours and this is similar to the asexual parasite clearance times. However, for patients who had gametocytes emerge during or following treatment, some patients from all treatment arms had gametocyte clearance times of less than 50 hours (Table 42). Irrespective of the treatment arm, 13 patients had gametocytes emerge following treatment initiation (time to emergence range: 6 to 72 hours), and their gametocyte clearance time ranged between 6 hours and >28 days.

Table 41: Patients with gametocytes present at baseline

Treatment Group	Patient ID number	Baseline Gametocyte Count*	Time to Gametocyte Clearance	Asexual PCT
Coartem (n=4)	14	7	30 hours	24 hours
	87	25	48 hours	12 hours
	150	141	21 days	30 hours
	154	801	Gametocytes present at day 28 (count=34)	24 hours
Lumefantrine capsules (n=1)	71	1039	21 days	66 hours
Lumefantrine tablets (n=4)	67	15	14 days	42 hours
	83	44	28 days	54 hours
	90	178	28 days	42 hours
*The gametocyte count seems unusually low, it is unknown if the number presented in the dataset refers to the number of gametocytes counted per slide (or 200 WBCs) or if this is truly the parasite density (i.e. gametocytes per µL)				

Table 42: Patients with gametocytes which emerged after baseline.

Treatment	Patient ID number	Gametocyte Count* (Hour reported)	Time to gametocyte Clearance	Asexual PCT of patient (hours)
Coartem (n=2)	22	2 (72)	18 hours	36
	76	6 (24)	12 hours	24
Lumefantrine capsules (n=6)	13	14 (12)	6 days	48
	73	69 (60)	11 days	60
	85	26 (72)	18 hours	72
	86	18 (24)	13 days	42
	93	114 (42)	12 days	60
	129	133 (36)	Gametocytes present at Day 28 (count=199)	66
Lumefantrine tablets (n=5)	3	8 (6)	3.5 days	48
	53	33 (54)	19 days	60
	65	12 (30)	6 hours	36
	128	8 (18)	13 days	72
	133	12 (30)	6 hours	42

4.1.3 Study 025 (Protocol 5669701025)

Study 025 was a 3-arm, randomized, double blind, parallel group trial which compared 4 doses of Coartem with 6 doses in adult and pediatric patients with *P. falciparum* infection and baseline parasitemia of >500 parasites per µL. The objectives were to evaluate safety, pharmacokinetics, and efficacy at two sites in Thailand (Hospital of Tropical Diseases, Mahidol University, Bangkok, Thailand and MaeLa Shoklo Malaria Research Unit, Mae Sot, Thailand). At the Bangkok center,

patients were admitted for in-patient observation over the 28 day trial whereas patients at the MaeLa facility were examined daily on an out-patient basis during the first week, then weekly until day 28; these patients were also followed on day 63. Dosing of each treatment occurred as follows: Arm 1 (120 patients) received 4 doses (480 mg lumefantrine / 80 mg artemether per dose) over 48 hours (hours 0, 8, 24, 48 with placebo at hours 36, 60, 72 and 96); Arm 2 (118 patients) received 6 doses (480 mg lumefantrine / 80 mg artemether per dose) over 60 hours (hours 0, 8, 24, 36, 48, 60 with placebo at hours 72 and 96). Arm 3 (121 patients) received 6 doses over 96 hours (hours 0, 8, 24, 36, 48, 60, 72 and 96). This trial compares the Coartem 4 dose with 6 dose regimens. It should be noted that the applicant has sought approval of a 6 dose regimen. The duration of the trial was 28 days and the efficacy endpoints were PCT, the cure rates on days 2, 3, and 28, as well as anti-gametocyte activity. Microbiological evaluations included parasitological measurements, genotyping, and *in vitro* sensitivity measurements. Microscopic examination of thick smears is considered to be an acceptable method for the diagnosis of malaria and for determining antimalarial drug efficacy. Genotyping by polymerase chain reaction (PCR) was used by the applicant to differentiate recrudescence from a new infection. The PCR assay is considered to be an experimental procedure and its use in differentiating recrudescence from new infection is not standardized.

Parasitological Measurements

Parasitologic diagnosis of patients enrolled in the clinical trials was made by examination of blood smears. At screening, up to 20 thick film fields were examined to identify asexual forms of *P. falciparum*; if asexual forms were found, 200 thick film fields were then examined for species other than *P. falciparum.* At the Bangkok center, parasite counts were performed on Giemsa stained thick films every 12 hours until parasite clearance was reached, followed by once daily until day 8, and then weekly until day 28 (i.e., days 15, 22 and 28). If infection reemerged, daily parasite counts were reinstated. In order for a slide to be declared negative, a total of 200 fields per slide were examined. At MaeLa site, parasite counts were performed on Giemsa stained thick films daily until parasite clearance was reached and then weekly until day 28 as well as a day 63 follow-up. At the Bangkok center, counting was based on the number of parasites per 200 leukocytes; if a low number of parasites were counted then the number of leukocytes was extended to 500. At MaeLa, counting was based on the number of parasites per 500 leukocytes. At both sites, parasite density was calculated as stated above for Study 023. If gametocytes were seen during examination of slides, a gametocyte count was performed until 1000 WBCs were counted. If the number of gametocytes per 1000 WBCs was less than 5, then 2000 WBCs were counted. There is no mention of quality control for parasitological quantification and it appears that only one slide per patient was examined.

Genotyping

Genotyping by polymerase chain reaction (PCR) was used by the applicant to differentiate recrudescence from new infection. The PCR assay is considered to be an experimental procedure and its use in differentiating recrudescence from new infection is not standardized. Though the applicant was requested to submit the details of the PCR method used and the performance characteristics of the assay in the actual laboratory where testing was done at the time of the pre-NDA meeting, the information was not included in the NDA submission. However, only details of the assay were provided by the applicant in response to a request by the Division (letter date July 9, 2008). Genotyping by the PCR assay used in this study was performed at the Shoklo Malaria Research Unit, Bangkok. The assay was based on repeat copy number polymorphisms of 3 loci:

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Primer sequences for MSP-1 and MSP-2 used for this study are those which were designed by Ranford-Cartwright *et al.,*[34] in a study which genotyped the oocyst stage of the parasite lifecycle. Primers for GLURP were designed by Paul *et al.,*[35] in a study which also genotyped the oocyst stage (Table 43). The Ranford-Cartwright study determined the genotype of *P. falciparum* oocysts following fertilization and was successful in genotyping 76.6 and 80.6% of the oocysts for the MSP-1 and MSP-2 genes, respectively. The study by Paul *et al.,*[35] references the primers used in the Ranford-Cartwright study and states that all of the primers are specific to *P. falciparum* and would not cross react with *P. vivax, P. ovale* or *P. malariae* DNA. However, the applicant does not state where the primers were synthesized or whether their purity and sequence was confirmed.

Table 43: Primer sequences used in PCR genotyping

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The applicant has not provided any information supporting the standardization and performance of the assay in the laboratory where patient blood samples were tested. For example, no evidence is given on primer efficiency and many aspects of the assay such as sensitivity, specificity, the reliability of control strains used either to monitor assay performance day to day (reproducibility), or operator to operator. There was no reported sequencing of the PCR products to confirm their identity. It remains unknown if nonspecific products were noted. Additionally, since PCR assays are inherently prone to environmental contamination, results of these analyses are germane to acceptance of results.

While the applicant does state the lower limit of detection of the assay, no data were provided to support this claim. It would be particularly helpful, given the high frequency of mixed infections due to different strains of *P. falciparum*, to perform a limiting dilution assay with mixed cocktails of different strains present in different proportions to determine the utility of PCR in differentiating recrudescence from new infection in patients infected with more than one strain of *P. falciparum*. Mixed infections of different *P. falciparum* strains which have a widely disproportionate multiplicity of infection (MOI) may be subject to misidentification of the strain with the lower MOI. Additionally, asexual forms of *P. falciparum* may be sequestered and not present in the peripheral blood during a significant part of the erythrocytic cycle, consistency in being able to identify all infecting strains at a single time point such as baseline may be impossible due to asynchrony of the infecting strains[36,37] .

Blood was obtained at baseline and upon reappearance of parasites by a finger prick. Blood collection was performed at either study center by spotting 30 µL of blood on **(b) (4)** chromatography paper for subsequent DNA extraction and analysis, all other steps of the assay were performed at the Shoklo Malaria Research Unit. **(b) (4)**

An initial PCR amplification with outer primers was followed by a nested PCR on the amplification product for increased specificity. The size of the nested PCR products was analyzed by agarose gel electrophoresis and a comparison of PCR product sizes between the baseline isolate and the isolate

obtained at reappearance was made to determine if the genotype profiles differed. The agarose concentration for the gel used to analyze each gene was optimized to allow for the separation of the different sized products to be apparent. The DNA product(s) amplified using the primers for MSP-1 were electrophoresed through gels containing 1.75% Seakem agarose plus 0.75% NuSieve agarose (w/v). For MSP-2 and GLURP, the DNA product(s) were electrophoresed through 1.5% Seakem agarose plus 0.5% NuSieve agarose (w/v). The size of the products was extrapolated using linear regression from a curve generated with the migration distance of a DNA ladder. Differentiating the band lengths was accomplished by classifying each band to a specific 40 bp "bin" for MSP-1 and MSP-2 and 60 bp bins for GLURP. In other words, products which differed by > 40 bp would be classified as separate MSP-1 or MSP-2 products (or bins) when compiling a profile of a specific isolate. This method was used in the study by Paul *et al.*,[35] for preparation of GLURP primers. Mixed infections, due to different strains, of *P. falciparum* have the potential to profile with multiple alleles (i.e., product sizes) for each gene. The applicant concedes that the possibility of primer bias for a particular genotype over another in a mixed strain infection exists. The possibility of primer bias may contribute to falsely classifying a mixed infection as a monoinfection particularly in cases of mixed infections with a broad range of strain specific parasite densities. The genotyping analysis of the patients with samples from the Bangkok center revealed that 54% of the patients who experienced reappearance of parasites were initially infected with mixed strains of *P. falciparum* (7 of 13 patients).

Another potential source of error lies in the inherent variability of using linear regression to estimate the fragment size of electrophoresed PCR products. There is a potential to incorrectly categorize a recrudescent infection as a new infection, for example, in a case where what is in reality an identical fragment being allocated to a different "bin" on different gel runs because the variability of linear regression may estimate the fragment length to be in a particular "bin" on one run and in its adjacent bin on another run. An actual example of this phenomenon is highlighted by data presented in Study 028 below. The applicant states that the lower limit of detection of the assay is 1 parasite/μL, though no data to support this claim were given. The applicant does concede that in a mixed infection, one strain may be below the lower limit of detection in the baseline assay and survive treatment, its emergence later may falsely be attributed to a new infection. The applicant has included a table listing the size ranges of the allelic classes used in this study and are shown in Table 44.

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Table 44: Size range "bins" used to classify product sizes after PCR genotyping

Allele code	MSP-1	MSP-2	GLURP
1	(b) (4)		
2	(b) (4)		
3	(b) (4)		
4	(b) (4)		
5	(b) (4)		
6	(b) (4)		
7	(b) (4)		
8	(b) (4)		
9			(b) (4)
10			(b) (4)
11			(b) (4)
12			(b) (4)

The authors have stated that the variability within MSP-2 was done first to determine if an isolate at the time of reappearance differed from the baseline isolate. If MSP-2 alleles were different, the isolate would be considered a new infection. If the MSP-2 gene profile was the same, MSP-1 and GLURP profiling were performed to confirm whether the isolate had the same profile as that obtained at baseline. If MSP-1 and GLURP differed from baseline, the isolate would be considered a new infection.

Given that twelve individual PCR results must be free of error to categorize an infection as recrudescent [2 samples (baseline & reappearance) x 3 genes (MSP-1, MSP-2, GLURP) x 2 PCRs (primary and nested) = 12], the odds that contamination, mislabeling or misinterpretation will alter these results are equally increased. Such a situation may potentially lead to over-reporting new infections (false negatives). Lastly, in order to gain even more confidence in the results of this method, the Division requested that actual gel results be provided for each patient tested. These results have been remitted to the Division. However, the gels have no patient identifications for an independent corroboration of results.

***In vitro* sensitivity testing**

Genotypically confirmed recrudescent isolates from patients treated with Coartem were processed for measuring *in vitro* drug sensitivity. Two of these isolates were stated to be recrudescent and 5 as new infections by PCR genotyping. These parasites were cryopreserved and/or set up in an *in vitro* culture or directly cultured in the presence of antimalarial drugs; this was dependent upon the parasite density at time of receipt of the sample. If the density was >100 parasites per 500 WBC, then a packed RBC pellet was cryopreserved in Tyrode's buffer and dimethylsulphoxide and stored in liquid nitrogen. Samples with lower parasite densities were set up for *in vitro* culture by placing the infected RBCs in RPMI medium with 10% heterologous patient serum. Once an optimum 0.5% parasitemia was achieved, each isolate was tested in a sensitivity assay conducted at the Armed Forces Research Institute of Medical Science in Bangkok. No information is provided that determined whether the method used to preserve or expand the isolate had an effect on the susceptibility assay. A micro dilution radioisotope technique by the incorporation of ^{3}H-hypoxanthine was utilized to determine the isolate's sensitivity to lumefantrine, artemether, quinine, mefloquine, and artesunate by the method of Webster *et al.*,[38] Each culture was performed in duplicate and a serial 2 fold dilution of each drug over

7 concentrations was tested. The 96 well microtiter plates containing each drug and the parasites were incubated for 24 hours at 37º C prior to the addition of 0.625 μCi (^{3}H)-hypoxanthine. The experiments determined the IC_{50} value of the drug as defined by the drug concentration needed to inhibit the uptake of (^{3}H)-hypoxanthine by the erythrocytic parasites. Baseline isolates from the same patient were not tested.

Parasitological Findings

Asexual Parasites

The intent to treat population consisted of 359 patients, while the per protocol population consisted of 306 patients (Table 45). In the PP population, there were 104 patients in Arm 1 (Coartem 4 dose regimen), 96 in Arm 2 (Coartem 6 doses over 60 hours) regimen, and 106 in Arm 3 (Coartem 6 doses over 96 hours). The baseline parasitemia range was 290 to 464,880 per μL with a median of 8840/μL. The results show the 6 dose regimens of Coartem were equivalent to the 4 dose regimen in parasite clearance time. The 28 day cure rates were better in patients treated with the 6 dose regimens (Arms 2 and 3) of Coartem compared to the 4 dose regimen (Arm 1). Overall, while the 4 dose regimen is effective in clearing parasites, the 6 dose regimen is more effective in achieving radical cure and lowering incidence of recrudescence in Thailand. All treatment failures, regardless of the treatment arm, can be classified as RI according to WHO criteria (Table 46).

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Table 45: The clearance of *P. falciparum* over time in response to different dosing regimens of Coartem

Parameter		Arm 1 Coartem 4 doses	Arm 2 Coartem 6 doses (60 hours)	Arm 3 Coartem 6 doses (96 hours)
Number of patients	**ITT**	120	118	121
	PP	104	96	106
Baseline parasitemia/µL [median (range)]	**ITT**	11891 (381 – 199980)	6276 (415 – 195735)	7480 (290 – 464880)
	PP	13080 (410 – 199980)	6276 (415 – 189740)	8050 (290 – 464880)
% patients in which parasites are cleared by 24 hours	**ITT**	21.7	25.4	22.3
	PP	21.2 n	22.9	20.8
% patients in which parasites are cleared by 48 hours	**ITT**	73.3	76.3	75.2
	PP	73.1	76.0	76.4
% patients in which parasites are cleared by 72 hours	**ITT**	99.2	99.2	96.7
	PP	99.0	97.9	97.2
% Cure rate at 7 days	**ITT**	100	100	100
	PP	100	100	100
% Cure rate at 14 days	**ITT**	100	99.1	100
	PP	100	99.0	100
% Cure Rate [28 days (Uncorrected)]	**ITT**	70.8	81.4	86.0
	PP	80.7	96.9	98.1
% Cure Rate [28 days (Corrected)]	**ITT**	73.3	82.2	87.6
	PP	83.7	96.9	99
PCT [mean hours (range)]	**ITT**	43.0 (18 – 72)	43.4 (17 – 166)	43.6 (18 – 90)
	PP	43.1 (18 – 72)	43.8 (17 – 166)	43.6 (18 – 90)
Recrudescence [number of patients (Uncorrected)]	**ITT**	20	4	2
	PP	20	3	2
Recrudescence [number of patients (Corrected)]	**ITT**	17	3	1
	PP	17	3	1

Note: For patient 46, the PCT was censored at hour 0, upon review of parasite counts, the PCT was shown to be 56.9 and was changed in the "eff" dataset for calculations. Calculations for parasite reduction at 24, 48 and 72 hours were not performed since the times of blood sampling were variable for each patient and few patients actually had samples taken near each of those time points.

Table 46: Treatment failures, in the ITT population, at day 28 as classified by the World Health Organization (based on uncorrected cure rates)*

Treatment Group (dose)	R-I	R-II	R-III
Coartem 4 doses	20	0	0
Coartem 6 doses (60 hours)	4	0	0
Coartem 6 doses (96 hours)	2	0	0

*WHO definitions of treatment failure:
R-I= clearance of asexual parasitemia within 7 days, followed by recrudescence
R-II=marked reduction of asexual parasitemia but no clearance (asexual parasite counts of <25% of baseline within 48 hours after initiation of treatment but no, or only temporary clearance of asexual parasitemia within 7 days
R-III=no marked reduction of asexual parasitemia (asexual parasite counts remain >25% of baseline at 48 hours, or actually rise above baseline levels at 48 hours without clearance of asexual parasitemia within 7 days.

Genotyping Analysis

Irrespective of the dosage regimen, there were 26 patients with reappearance before day 28, and 6 patients who had reappearance after day 28. Of the 32 patients with paired isolates, PCR was done on 31 patients. Of these 31 patients, 22 were considered to be recrudescent patients, 9 new infections and one subject (# 212) was not tested by PCR (for the purpose of this review this patient was conservatively categorized as recrudescent; Table 48). As expected, given the in-patient design of the study conducted at Bangkok, all patients with reappearance of parasites were found to be recrudescent (all patients in Table 47 with Subject IDs between 4 and 112). This gives some support to the ability of the method to detect a majority of mixed infections as none were falsely classified as a new infection due to a minority genotype which was undetectable at baseline surviving treatment and emerging later.

At the MaeLa site, 13 patients had parasites reappear by day 28. Of these 13 patients, 8 were recrudescent, while 5 were considered new infections. Based on a follow-up after day 28, 6 more patients had reappearance of parasites of which 2 cases were classified as recrudescence and 4 as new infection, however due to the appearance lying outside of the 28 day trial window, these results are not included in any analysis of efficacy.

The applicant has stated that if the results of the assay failed to produce a result due to failure of the PCR to produce a product or if multiple bands were present at 2 or more gene loci which would make strain identification impossible, the PCR results were considered indeterminate. No PCR results were classified as indeterminate in this study.

A possibility exists of a patient being re-infected with the same strain. A probability analysis was performed to determine whether a patient with a specific genotype at baseline who had reappearance with the same genotype following treatment could be conclusively classified as recrudescent. The applicant has stated that given the most common genotype observed [MSP-1 allele 3, MSP-2 allele 6 and GLURP alleles 7 and 9 (both occurred with the same frequency)], the chance that a patient presenting with the same genotype at baseline and then presenting with the same genotype due to a new infection, and not recrudescence was < 5%. Therefore, all paired isolates which were identical at baseline and reappearance could be classified as recrudescence with >95% confidence. The applicant has stated in the summary report that the frequency of the three different genotypes detected in the samples are shown in a table. However, this table was not included in the report. Furthermore, the

applicant comments that it is preferable to have at least 100 samples analyzed to determine profile frequency; it is unknown if 100 samples were used to determine genotype profile frequency at this geographical region in this report, but the applicant's conservative approach to classifying two identical profiles obtained at baseline and reappearance as recrudescence is appropriate. It would be beneficial if genotyping was performed on all baseline isolates to determine if a particular genotype was more likely to be resistant to treatment. The applicant stated that the WHO only recommends performing this analysis if treatment failure exceeds 5%.

Given the inherent complications of the assay and the absence of performance characteristics and quality control, results based of PCR genotyping should not be used for evaluating drug efficacy.

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Table 47: Genotyping in 32 patients with paired isolates at baseline and recrudescence

Subject ID	Baseline					Recrudescence					
	Treatment group	Parasite Count	MSP-1 allele bin	MSP-2 allele bin	GLURP allele bin	Parasite Count	Day	MSP-1 allele bin	MSP-2 allele bin	GLURP allele bin	New infection or Recrudescence
Bangkok, Thailand (n=13) by day 28											
4	Coartem 4 dose	23000	2	6	5	1786	13	2	6	5	Recrudescence
7	Coartem 4 dose	38080	3	7	4, 8	69	16	3	7	4, 8	Recrudescence
23	Coartem 6 (60hr)	49440	3	6	7	1388	19	3	6	7	Recrudescence
33	Coartem 4 dose	9020	3	4	9	128	16	3	4	9	Recrudescence
38	Coartem 4 dose	158360	2	6	8	1	19	2	6	8	Recrudescence
52	Coartem 4 dose	43760	3	2, 6	8, 9	360	19	3	2	8	Recrudescence
76	Coartem 6 (96hr)	42030	2	0, 4	7, 9	38	13	2	0, 4	7, 9	Recrudescence
82	Coartem 4 dose	24840	3	2, 6	11, 9	80	17	3	2	11	Recrudescence
84	Coartem 4 dose	23400	0	2, 7	11, 12	195	15	0, 2	2, 7	11, 12	Recrudescence
86	Coartem 6 (60hr)	4390	3	4	7	150	12	3	4	7	Recrudescence
89	Coartem 4 dose	31920	3, 4	2, 6	10	579	14	3, 4	2, 6	10	Recrudescence
90	Coartem 4 dose	13920	3	5	7	1335	21	3	5	7	Recrudescence
112	Coartem 4 dose	25080	3	7, 8	10	1544	21	3	7	10	Recrudescence
MaeLa, Thailand (n=13) by day 28											
181	Coartem 6 (60hr)	1209	1, 1	5	10	28	13	1, 1	5	10	Recrudescence
190	Coartem 4 dose	76641	1, 2	2, 6	4, 7, 8	32002	20	1	2	8	Recrudescence
211	Coartem 4 dose	49851	2	2	5	350	13	2, 3	4	8	New infection
212**	Coartem 4 dose	140747	NA	NA	NA	451	21	NA	NA	NA	Recrudescence
237	Coartem 4 dose	89490	1	1, 7	8, 9, 6	432	20	1	1	8, 9	Recrudescence
299	Coartem 4 dose	37479	3	2	9	312	20	3	2, 8	9, 10	Recrudescence
316	Coartem 4 dose	24919	2	5	8	87041	20	2	5	8	Recrudescence
380	Coartem 4 dose	15976	1, 2	2	6, 7	173	20	1	2	6	Recrudescence
410	Coartem 4 dose	3311	1, 2	3, 4	8	422	23	1	3	8	Recrudescence
243*	Coartem 6 (60hr)	451	1, 2	1, 5	7, 9, 11	1185	25	0, 2	1, 5	6, 8, 9	New Infection
268	Coartem 4 dose	26225	2	2, 3	4, 6, 8	623	27	2, 3	2, 3, 6	9, 12	New Infection
284	Coartem 6 (96hr)	6542	4	3, 7	9, 10, 11	386	27	3	3	11	New Infection
296	Coartem 4 dose	43960	4	2, 8	9, 10	1827	20	2	11	8	New Infection
MaeLa, Thailand (n=6) after day 28											
203	Coartem 4 dose	36097	2	2	10	46007	35	2	2	10	Recrudescence
249	Coartem 6 (96hr)	48532	2, 2	2, 7	4, 8, 8	146386	48	2	2	8	Recrudescence
187	Coartem 6 (60hr)	2054	1, 2	2, 5, 6	6, 9	5280	40	1	2	8	New Infection
206	Coartem 4 dose	4579	1	4	10	66618	40	1	1	8	New infection
382	Coartem 6 (96hr)	918	1	5	10	25673	40	1	4	6	New Infection
391	Coartem 6 (60hr)	65312	2	1	7	42415	60	2	5	7	New Infection

*Subject 243 was incorrectly classified as a new infection in the applicant's analysis, it remains possible that in addition to a new infection, a portion of the genotypes seen could be recrudescence of a strain of type 2-5-9 or 2-1-9 which was present at baseline.
*** Subject 212 had reappearance of parasites, no PCR was performed on this subject and in the efficacy analysis will be conservatively classified as recrudescent.

Table 48: Results of PCR genotype analysis on paired patient blood samples

Site	New infection or Recrudescence	Arm 1 (4 x4 regimen)	Arm 2 (6 x 4 over 60 hours)	Arm 3 (6 x 4 over 96 hours)	Total
MaeLa	New infection	4	3	2	9
	Recrudescence	7	1	1	9
	PCR not done	1	0	0	1
Bangkok	Recrudescence	10	2	1	13
Total		22	6	4	32

In vitro sensitivity of recrudescent clinical isolates

Parasites were isolated from the blood of 7 patients who experienced reappearance of parasitemia within the 63 day follow-up period to determine whether genotypically confirmed recrudescent isolates which had been exposed to Coartem *in vivo* had decreased sensitivity to the drug *in vitro*. Two of these isolates were confirmed to be recrudescent and 5 were determined to be new infections by PCR genotyping. The results of the analysis showed that artemether IC_{50} values were similar for isolates characterized as recrudescent or new infections by genotyping. It is interesting to note that 3 of the 5 patients with new infections had isolates with high lumefantrine IC_{50} values of > 100 ng/mL (Table 49). Testing of baseline isolates was not included and chloroquine resistance was apparently not determined even though the study took place in an area of chloroquine resistance.

Table 49: The *in vitro* sensitivity of 5 new infections and 2 recrudescent infections of *P. falciparum* to 5 drugs following treatment with Coartem

Patient	Reinfection category	IC_{50} (ng/mL)				
		Artemether	Artesunate	Lumefantrine	Quinine	Mefloquine
187	New	4.42	2.92	>100	347.35	59.96
206	New	8.54	8.85	>100	938.78	>100
243	New	3.86	2.13	>100	729.6	58.31
382	New	1.75	1.52	32.61	376.29	30.53
296	New	-	3.22	-	>1000	43.98
316	Recrudescent	7.75	2.04	37.5	166.43	54.33
249	Recrudescent	5.44	1.87	25.25	214.8	16.25
Please see Table 47for the genotype of the reappearing isolate for each patient.						

P. vivax co-infection

At baseline, 20 patients (Arm 1: n=7; Arm 2: n=7; and Arm 3: n=6) were co-infected with *P. falciparum* and *P. vivax*. The mean (range) time to *P. vivax* clearance was 20.7 (11 – 42.5), 22.7 (17 – 41), and 27.35 (19 – 43.2) hours for the Coartem Arms 1, 2, and 3, respectively. *P. vivax* reappeared in 10 of these 20 patients at some point during the trial (Table 50). The time of reappearance ranged from days 15 to 39. Additionally, 45 patients had appearance of *P. vivax* after baseline. The results do not support that Coartem has activity against the hypnozoite (liver) stage of the parasite; relapse may occur. It should be noted that 4 of the patients who had *P. vivax* coinfection also experienced reappearance of *P. falciparum* parasites and underwent genotyping analysis. It is unknown if the presence of *P. vivax* affected the outcome of the genotyping analysis.

Table 50: Summary of patients who were co-infected with *P. vivax* and *P. falciparum*

Coartem Regimen	Baseline			After initiation of treatment			
	Number of patients with *P. vivax*	*P. vivax* clearance time in hours [Mean (range)]	Number of Patients who relapsed	Number of patients with *P. vivax*	Day of emergence [Median (range)]	*P. vivax* clearance time time in hours [Mean (range)]*	Number of patients who relapsed
4 x 4 (48hr)	7	20.7 (11 – 42.5)	4	17	27 (10 – 62)	184 (23 – not cleared)	0
6 x 4 (60hr)	7	22.7 (17 – 41)	4	18	27 (1 – 67)	165 (24 - not cleared)	0
6 x 4 (96hr)	6	27.35 (19 -43.2)	3	11	36 (21 – 63)	341 (144 - not cleared)	0

* The clearance time for patients with *P. vivax* emerging after baseline is artificially high due to infrequency of parasite counts during this period of the trial and many patients did not have a confirmed clearance because they still had *P. vivax* parasitemia at the last visit.

Gametocytes

A total of 46 patients presented with gametocytes at baseline and an additional 25 patients had emergence of gametocytes at some point thereafter (Table 51). It is unknown if the difference between groups in patients who presented with gametocytes at baseline and during the trial is significant, but it is noted that the Coartem 6 dose (60 hours) arm had less patients, at baseline and after initiation of treatment, than the other two arms. The median time to gametocyte clearance was less for the 4 dose regimen than for either 6 dose regimen, but all were less than 7 days.

Table 51: Summary of gametocyte data for Study 025

Group	Number of patients with gametocytes	Gametocyte count at baseline or emergence [Mean (range)]	Gametocyte clearance time [Median (range)]	Asexual parasite clearance time in hours [Median (range)]
Arm 1 (Coartem 4 doses)	At baseline (n=18)	486 (17 -4220)	87 (19 – 472)	44.1 (20.25 -71.67)
	After initiation of treatment (days 1 – 28) (n=11)	1309 (20 – 8264)	48.5 (23 – 336)	44 (20.25 – 66.67)
Arm 2 [Coartem 6 doses (60 hours)]	At baseline (n=9)	542 (13-1366)	162 (44 – 672)	42.1 (19.85 – 68)
	After initiation of treatment (days 1 – 28) (n=3)	33 (15 – 49)	144 (96 – 144.7)	39 (20 – 45)
Arm 3 [Coartem 6 doses (96 hours)]	At baseline (n=19)	686 (20 -3960)	114 (9 – 420)	44.1 (18.3 – 89.5)
	After initiation of treatment (days 1 – 28) (n=11)	36 (1 – 57)	36 (12 – 168)	45 (18 – 89)

4.1.4. Study 026 (Protocol 5669701026)

Study 026 was a randomized, open label, parallel group trial which compared 6 doses of Coartem (20 mg artemether/120 mg lumefantrine over 3 days) to mefloquine + artesunate (MAS) in 200 patients with *P. falciparum* infection to evaluate the safety and efficacy at two sites in Thailand (Hospital of Tropical Diseases, Mahidol University, Bangkok, Thailand and MaeLa Shoklo Malaria Research Unit, Mae Sot, Thailand). These are the same sites as for Study 025. This trial also enrolled pediatric patients. Coartem was administered over 3 days (hours 0 and 8, then twice daily on days 2 and 3). Since efficacy was shown to be comparable in the 6 dose regimen over 60 hours versus 96 hours in Study 025, a 3 day regimen was chosen as it was thought this would increase compliance. Patients in the MAS arm received 6 doses of artesunate (4 mg/kg) once daily for 3 days and mefloquine (25 mg/kg) as a split dose of 15 mg/kg plus 10 mg/kg on days 2 and 3. Patients were followed for 29 days and the primary efficacy endpoints were the 28 day cure rate and the proportion of patients with negative slides on days 2, 3, and 4. The effect on gametocyte count was also reported. At the Bangkok center, patients were admitted for in-patient observation over the 28 day trial. Patients at the MaeLa facility were examined daily on an out-patient basis during the first week, then weekly until day 29. Some of these patients were also seen for follow-up on days 36 and 43.

Parasitological Measurements
At screening, up to 200 high powered fields of a thick film were examined for the presence of *P. falciparum* asexual parasites. If asexual forms were observed, an additional 200 high power fields were examined for species other than *P. falciparum*. Baseline counts were based on counting the number of parasitized erythrocytes per 200 WBCs. If this number was low, then counting was extended to 500 WBCs. Parasite density was calculated according to the formula described for Study 025. During the trial, parasite counts were performed on Giemsa stained thick films daily until parasite clearance was reached, parasite counts were then taken weekly (days 8, 15, 22 and 29). In order for a slide to be declared negative, a total of 200 fields per slide were examined. If infection reemerged, daily parasite counts were reinstated.

If gametocytes were seen during examination of slides, a gametocyte count was performed until 500 WBCs were counted. If the gametocyte count was less than 5 gametocytes per 500 WBCs, the count was extended to 2000 WBCs. If *Plasmodium* species other than *P. falciparum* were seen at any time, the species was noted and a count was performed.

Genotyping
Though the efficacy results are expressed as uncorrected cure rates, PCR was utilized to distinguish reinfection from recrudescence upon reappearance of parasites in 3 patients who received Coartem at the MaeLa Center. All steps of the PCR assay were performed at the Shoklo Malaria Research Unit and are the same as discussed above (Study 025).

Parasitological Findings
Asexual Parasites
Patients were to be enrolled if their baseline parasitemia was >500 parasites per μL. However, two patients had baseline counts of < 500 parasites per μL and were included in efficacy analysis. The baseline parasitemia range was 264 - 254,490 parasites/μL with a median of 9,269/μL. The intent to

treat population (n=200) consisted of 150 patients randomized to Coartem and 50 patients randomized to MAS, while the per protocol population consisted of 181 patients (134 in the Coartem and 47 in the MAS arm). Results in Table 52 show that the 6 dose regimen of Coartem was equivalent to the MAS regimen in parasite clearance time. However, MAS was better than Coartem in achieving complete cure without recrudescence based on uncorrected cure rates (0 versus 5 patients, respectively). All treatment failures can be classified as RI according to WHO criteria (Table 53).

Genotyping Analysis
Of the 5 patients classified as recrudescent based on positive blood smears, PCR genotyping concluded that 2 were recrudescent and 1 was a new infection (Table 54). However, PCR was not done on 2 of the 5 patients. As discussed for Study 025 the results of the PCR assay should not be used to evaluate efficacy of the drug.

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Table 52: The clearance of *P. falciparum* over time in Study 026

Parameter		Coartem 6 doses	MAS
Number of Patients	**ITT**	150	50
	PP	134	47
Baseline parasitemia/µL [median (range)]	**ITT**	9374 (264 – 254490)	5284 (625 – 177840)
	PP	8934 (264 – 254490)	5195 (625 – 177840)
% parasite reduction at 24 hours [median(range)]	**ITT**	99.1 (-1952 – 100)	99.7 (73 – 100)
	PP	99.1 (-1952 – 100)	99.7 (73 – 100)
% patients in which parasites are cleared by 24 hours	**ITT**	20.4	27.1
	PP	22.0	24.4
% patients in which parasites are cleared by 48 hours	**ITT**	78.9	77.1
	PP	78.0	77.8
% patients in which parasites are cleared by 72 hours	**ITT**	94.6	93.8
	PP	94.7	93.3
% Cure rate at 7 days	**ITT**	99.3*	100
	PP	100	100
% Cure rate at 14 days	**ITT**	99.3*	100
	PP	100	100
% Cure Rate [28 days (Uncorrected)]	**ITT**	86	94
	PP	96.2	100
% Cure Rate [28 days (Corrected)]	**ITT**	86.7	94
	PP	97	100
PCT [mean hours(range)]	**ITT**	42.3 (17.6 – 96) n=150	41.5 (16.9 – 72) n=50
	PP	41.8 (17.6 – 96) n=134	42.3 (16.9 – 72) n=47
Recrudescence [number of patients (Uncorrected)]	**ITT**	5	0
	PP	5	0
Recrudescence [number of patients (Corrected)]	**ITT**	4	0
	PP	4	0

Note: Since most patients did not have a count performed at 24 hours, the calculation for "% parasite reduction at 24 hours" was made with parasite counts between 18 and 30 hours with the count closest to 24 hours used per patient.
***** A lower cure rate for the Coartem arm at 7 and 14 days in the ITT population was due to patient 38 who never had any parasite counts taken. This patient was excluded from the determination of PCT.

Table 53: Treatment failures as classified by the World Health Organization (based on uncorrected cure rates)*

Treatment	R-I	R-II	R-III
Coartem 6 x 4	5	0	0
MAS	0	0	0

*WHO definitions of treatment failure:
R-I= clearance of asexual parasitemia within 7 days, followed by recrudescence
R-II=marked reduction of asexual parasitemia but no clearance (asexual parasite counts of <25% of baseline within 48 hours after initiation of treatment but no, or only temporary clearance of asexual parasitemia within 7 days
R-III=no marked reduction of asexual parasitemia (asexual parasite counts remain >25% of baseline at 48 hours, or actually rise above baseline levels at 48 hours without clearance of asexual parasitemia within 7 days.

Table 54: Results of PCR genotype analysis on paired patient blood samples

Site	Recrudescence vs. New infection	Coartem 6 x4	MAS
MaeLa	New infection	1	0
	Recrudescence	2	0
	PCR not done	2	0
	Total	5	0

***P. vivax* co-infection**

At baseline, 6 patients were seen to have mixed infections of *P. falciparum* and *P. vivax*; 5 and 1 for those patients in the Coartem and MAS arms, respectively (Table 55). The mean (range) time to *P. vivax* clearance was 20.1 (18.5 – 21.3) and 20 hours for Coartem and MAS, respectively. However, *P. vivax* reappeared in 2 of the 5 patients in the Coartem arm at a later time (day 28 and day 48). Additionally, 11 patients in the Coartem arm had appearance of *P. vivax* after baseline parasite counts were taken but before day 28, the median (range) day of emergence was day 23 (day 21 – 28). For these patients, the mean time to clearance was 194.7 hours, but measurements were only taken weekly for most patients so these time spans may be artificially high. Two of the 11 patients failed to clear *P. vivax* by the day on which the last blood smear was examined. It is unknown if those patients who cleared vivax parasitemia had relapse at a later time. No patients in the MAS arm were seen to have *P. vivax* infection beyond those seen at baseline. Additionally, 18 patients in the Coartem arm were seen to be infected with *P. vivax* after day 28.

Table 55: Summary of patients who were co-infected with *P. vivax* and *P. falciparum*

Treatment Arm	Baseline			After initiation of treatment			
	Number of patients with *P. vivax*	*P. vivax* clearance time in hours [Mean (range)]	Number of Patients who relapsed	Number of patients with *P. vivax*	Day of emergence [Median (range)]	*P. vivax* clearance time in hours [Mean (range)]*	Number of patients who relapsed
Coartem	5	20.1 (18.5 – 21.3)	2	11	23 (21 – 28)	194.7 (72 – not cleared)	0
MAS	1	20	0	0	NA	NA	0

* The clearance time for patients with *P. vivax* emerging after baseline is artificially high due to infrequency of parasite counts during this period of the trial and some patients did not have a confirmed clearance because they still had *P. vivax* parasitemia at the last visit.

Gametocytes

Of 14 patients presented with *P. falciparum* gametocytes at baseline, 10 were in the Coartem arm and 4 in the MAS arm (Table 56). An additional 7 patients had emergence of gametocytes after baseline (days 1 – 3) during the trial and 1 patient was found to have gametocytes on day 29. All patients in the Coartem arm had an initial gametocytemia of 12 – 1,414 gametocytes per µL except Patient 134 who had 67,870 gametocytes per µL. The median time to gametocyte clearance was 5.8 days for the Coartem regimen and 2.4 days for the MAS arm (Table 57). The lack of emergence of gametocytes after day 3 is probably more reflective of elimination of the asexual stage of the parasite due to drug treatment.

Table 56: Gametocyte presence at different time points during Study 026

Day	Number (%) of patients	
	Coartem (n=150)	MAS (n=50)
Baseline	10 (6.7%)	4 (8.0%)
Day 1	15 (10%)	4 (6.0%)
Day 2	15 (10%)	3 (4.0%)
Day 3	13 (8.6%)	2 (4%)
Day 4	11 (7.3%)	1 (2%)
Day 8	5 (3.3%)	0
Day 15	4 (2.6%)	0
Day 29	2 (1.3%)	0

Table 57: Summary of gametocyte data

Treatment Arm	Number of patients with gametocytes	Initial gametocyte count [Mean (range)]	Gametocyte clearance time in hours [Median (range)]	PCT in hours [Median (range)]
Coartem 6 doses	At baseline (n=10)	3992 (12 – 67870)	141.45 (43.3 –668)	45.97 (18 – 72)
	After initiation of treatment (day 1-28) (n=7)	283 (13 – 1414)	99.3 (46 – 503)	44 (18 – 67)
MAS	At baseline (n=4)	1867 (19 – 3944)	57.45 (18 – 92)	46.26 (42 – 48)
	After initiation of treatment (day 1-28) n=0	NA	NA	NA

4.1.5. Study 028 (Protocol 5669701 028)

Study 028 was a randomized, open label, parallel group trial which compared Coartem (n=164) to MAS (n=55) in patients, > 12 years of age, with *P. falciparum* infection to evaluate safety and efficacy and was conducted in Thailand (Hospital of Tropical Diseases, Mahidol University, Bangkok, Thailand). The dosing regimen for Coartem and MAS was the same as Study 026. The range of baseline parasitemia for the purpose of enrollment was not specified. The duration of the trial was 29 days and the primary efficacy endpoints were the PCT, 28 day cure rate, and the proportion of patients with negative slides on days 2, 3, and 4. Anti-gametocyte activity was also reported.

Parasitological Measurements
Patients were monitored by blood microscopy thrice daily for the first three days. Thereafter, a weekly visit was instated. If *P. falciparum* parasites reemerged, more frequent parasite counts were taken. At screening, up to 200 high powered fields of a thick film were examined. If asexual forms were found, 200 high powered fields were examined for species other than *P. falciparum*. Baseline counts were based on counting the number of parasitized erythrocytes per 200 WBCs. If this number was low, then counting was extended to 500 WBCs. Parasite density was calculated according to the formula given for Study 025. During the trial, parasite counts were performed on Giemsa stained thick films daily until parasite clearance was reached, parasite counts were then taken weekly (days 8, 15, 22 and 29). In order for a slide to be declared negative, a total of 200 fields per slide were examined. Gametocyte counting was performed as described for Study 026.

Genotyping Analysis
PCR was utilized to distinguish reinfection from recrudescence upon reappearance of parasites in the 7 patients who received Coartem. As for study 025, all steps of the assay were performed at the Shoklo

Malaria Research Unit. However, as stated above, the performance characteristics of the assay and results of quality control were not provided for an independent review.

Parasitological Findings

Asexual Parasites

The intent to treat population consisted of 164 patients randomized to Coartem and 55 patients randomized to MAS, while the per protocol population consisted of 155 Coartem treated patients and 53 MAS treated patients. The baseline parasitemia range was 13 – 436,050 parasites/μL with a median of 4,150 parasites/μL. As was also demonstrated for study 026, Coartem was equivalent to the MAS regimen in parasite clearance time (Table 58). Overall, the kinetics of clearance was also similar between both arms. MAS was better than Coartem in achieving complete cure without recrudescence (0 versus 7 patients, respectively). The median (range) day of reappearance was day 18 (days 17 – 29). All treatment failures can be classified as RI according to WHO criteria (Table 59).

Table 58: The clearance of *P. falciparum* over time in Study 028

Parameter		**Coartem 6 doses**	**MAS**
Number of patients	**ITT** (n=219)	164	55
	PP (n=208)	155	53
Baseline parasitemia/µL [median (range)]	**ITT**	2136 (13 – 436050)	5500 (21 – 207840)
	PP	1482 (13 – 436050)	5500 (21 – 207840)
% parasite reduction at 24 hours [median(range)]	**ITT**	99.9 (-17617 – 100)	99.9 (-321 – 100)
	PP	99.9 (-17617 – 100)	99.9 (-321 – 100)
% patients in which parasites are cleared by 24 hours	**ITT**	41.5%	32.7%
	PP	43.9%	33.9%
% patients in which parasites are cleared by 48 hours	**ITT**	92.1%	92.7%
	PP	93.5%	94.3%
% patients in which parasites are cleared by 72 hours	**ITT**	100%	100%
	PP	100%	100%
% Cure rate at 7 days	**ITT**	100%	100%
	PP	100%	100%
% Cure rate at 14 days	**ITT**	100%	100%
	PP	100%	100%
% Cure Rate [28 days (Uncorrected)]	**ITT**	90.2%	96.4%
	PP	95.5%	100%
% Cure Rate [28 days (Corrected)]	**ITT**	90.9%	96.4%
	PP	96.1%	100%
PCT [mean (range)]	**ITT**	30.0 (7 – 64)	29.7 (6.8 – 57.2)
	PP	29.0 (7 – 58.5)	29.1 (6.8 – 57.2)
Recrudescence [number of patients (Uncorrected)]	**ITT**	7	0
	PP	7	0
Recrudescence [number of patients (Corrected)]	**ITT**	6	0
	PP	6	0
Note: Since most patients did not have a count performed at precisely 24 hours, the calculation for "% parasite reduction at 24 hours" was made with parasite counts between 20 and 28 hours with the count closest to 24 hours used per patient.			

Table 59: Treatment failures as classified by the World Health Organization (based on uncorrected cure rates)*

	R-I	R-II	R-III
Coartem 6 doses	7	0	0
MAS	0	0	0

*WHO definitions of treatment failure:
R-I= clearance of asexual parasitemia within 7 days, followed by recrudescence
R-II=marked reduction of asexual parasitemia but no clearance (asexual parasite counts of <25% of baseline within 48 hours after initiation of treatment but no, or only temporary clearance of asexual parasitemia within 7 days
R-III=no marked reduction of asexual parasitemia (asexual parasite counts remain >25% of baseline at 48 hours, or actually rise above baseline levels at 48 hours without clearance of asexual parasitemia within 7 days.

Genotyping analysis

Six of the 7 patients were recrudescent, while 1 was considered a new infection (Tables 60 and 61). The applicant does not present the results as corrected cure rates in the efficacy analysis, though the difference is negligible since only 1 patient was determined to be a new infection. As discussed for Study 025, the potential for misallocation of a PCR fragment to a particular "bin" based on the inherent variability of linear regression in the estimation of product fragment length exists. This is highlighted by comparing patient 145 to patient 296. In the annexure to the study report presenting PCR results, the actual fragment lengths of the PCR products are given. For patient 145, the estimated lengths of the GLURP fragment amplified from the baseline blood sample and the blood sample taken at the time of parasite reappearance were 971 and 988 base pairs (bps), respectively. The difference in estimated fragment lengths was 17 bp. Both of these fragments were allocated to bin 10. This, along with the same bin classification of fragments obtained for MSP-1 and MSP-2, resulted in this reappearance being classified as recrudescence. For patient 296, a 17 bp difference was seen between the estimated fragment lengths for MSP-2 for those products amplified from the paired blood samples. In this case, however, due to the cutoff for bin classification, the baseline product was categorized as bin 8 (710 bp) and the fragment amplified from the reappearance sample was categorized as bin 9 (727 bp). This difference led to the classification of this patient's reappearance as a new infection. Gel results could not be located by the applicant for this study. In the absence of performance characteristics and quality control of this PCR assay, the results should not be used for differentiating recrudescence from new infection and presenting uncorrected cure rates in the package insert.

Table 60: Results of PCR genotype analysis on paired patient blood samples in patients treated with Coartem

Subject ID	Baseline					Recrudescence					
	Treatment group	Parasite Count	MSP-1 allele bin	MSP-2 allele bin	GLURP allele bin	Parasite Count	Day	MSP-1 allele bin	MSP-2 allele bin	GLURP allele bin	New infection or Recrudescence
145	Coartem	81650	2	6	10	23	16	2	6	10	Recrudescence
146	Coartem	8080	2	6	10	40	15	2	6	10	Recrudescence
159	Coartem	422400	3	7	7, 9	7180	19	3	7	7, 9	Recrudescence
166	Coartem	273	3	5	8	15030	27	3	5	8	Recrudescence
266	Coartem	25600	4	3	8	63	16	4	3	8	Recrudescence
277	Coartem	24440	3, 4, 5	4, 7	6, 10	704	20	4	4	6	Recrudescence
296	Coartem	16650	3	7, 8	7, 9	39	16	3	9	9	New Infection

Table 61: Results of PCR genotype analysis on paired patient blood samples

Result of PCR genotyping	Coartem 6 x4	MAS
New infection	1	0
Recrudescence	6	0
Total	7	0

***P. vivax* co-infection**

Table 62 summarizes the patients who were co-infected with *P. vivax* and *P. falciparum* during the trial. In patients with *P. vivax* at baseline, the mean time to *P. vivax* clearance was 21.6 and 20.8 hours for the Coartem and MAS arms, respectively. However, *P. vivax* recurred in 3 of the 16 patients in the Coartem arm at a later time (days 22, 24, and 28). Additionally, 3 patients in the Coartem arm had appearance of *P. vivax* after baseline parasite counts were taken but before day 28, the median (range) day of emergence was day 17 (day 1 and 19). Of these patients, the mean time to clearance was 66.5 hours from the time of emergence. All of the patients were successful in clearance of *P. vivax* except patient 102 (Coartem treated) who had reappearance of *P. vivax* and was still parasitemic at the time of the last blood smear examination. It is unknown if those patients who cleared vivax parasitemia relapsed at a later time. No patients in the MAS arm were seen to have *P. vivax* infection beyond those seen at baseline and there was no relapse in any patients following clearance.

Table 62: Summary of patients who were co-infected with *P. vivax* and *P. falciparum*

Treatment Arm	Baseline			After initiation of treatment			
	Number of patients with *P. vivax*	***P. vivax* clearance time in hours [Mean (range)]**	**Number of Patients who relapsed**	**Number of patients with *P. vivax***	**Day of emergence [Median (range)]**	***P. vivax* clearance time in hours (Mean)**	**Number of patients who relapsed**
Coartem	16	21.6 (7 – 40.3)	3	3	17 (1 – 19)	66.5*	1
MAS	7	20.8 (8 – 41.3)	0	0	NA	NA	0

*Parasitological measurements were taken less frequently at this point in the trial, so clearance time may be artificially high.

Gametocytes

The number of patients with gametocytes present at baseline and thereafter is summarized in Table 63. At baseline gametocytes were reported in 21 patients (14 in the Coartem arm and 7 in the MAS arm). An additional 18 patients (14 Coartem and 4 MAS arms) had emergence of gametocytes after baseline (days 1 – 28) during the trial. The time of emergence after baseline for MAS varied from days 2 to 14 and for Coartem days 1 to 28 (Table 64).

Table 63: Gametocyte presence during Study 028

Day	Number patients with gametocytes	
	Coartem	MAS
Baseline	14	7
Day 1	24	7
Day 2	19	4
Day 3	15	6
Day 4	13	4
Day 8	9	2
Day 15	0	1
Day 29	1	1

As shown in Table 64, a wide range of variation can be seen for both baseline gametocyte count and clearance time. When examining individual patient data, some correlation can be seen between the initial count and the time to clearance; all patients with an initial count >1000 gametocytes per µL had clearance times of > 300 hours. This correlation did not extend to those patients with low initial counts; e.g. a patient with an initial count of 6 had a clearance time of 207 hours whereas another patient with the same gametocyte density had a clearance time of 27 hours.

Table 64: Gametocyte clearance summary for Study 028.

Treatment Arm	Number of patients with gametocytes	Mean initial gametocyte count (range)	Gametocyte clearance time in hours [median (range)]	Asexual parasite clearance time in hours [median (range)]
Coartem 6 doses	At baseline (n=14)	1061 (25 – 8790)	126 (16 – 323)	32 (8 – 48)
	After initiation of treatment (day 1-28) (n=14)	52 (1 – 207)	24 (8 – 164)	32 (17 – 43)
MAS	At baseline (n=7)	190 (15 – 1100)	49 (6 – 328)	28 (8 – 50)
	After initiation of treatment (day 1-28) (n=4)	46 (20 – 74)	168 (8 – 646)	25 (24 – 32)

4.1.6. Study 2401

Study 2401 was an open label, multi-center, non-comparative study to evaluate the efficacy, safety and tolerability of Coartem (6 dose regimen over 3 days; same as for Study 026) in the treatment of uncomplicated *P. falciparum* malaria in non-immune travelers from Switzerland, Germany and Colombia and was distributed across 9 centers in Europe and Colombia. The initial enrollment of the

trial was 150 patients; a protocol amendment was devised which included an additional 15 patients for a "PK rich" study after the core trial had commenced. These patients received the same drug regimen and efficacy measurements were based upon the same criteria, therefore, for the purpose of this review both patient populations are combined (n=165).

The duration of the trial was 29 days and the primary efficacy endpoint was the 28 day cure rate. Other endpoints included the 7 day cure rate, PCT, gametocyte clearance time, and the incidence of early and late treatment failures. Early treatment failure was defined as development of severe malaria between days 1 and 7, day 2 parasitemia > day 0 count, day 3 parasitemia > 25% of day 0 count, symptomatic parasitemia between days 4 and 7 or parasitemia on day 7 (i.e. a positive smear). It will be difficult to determine some of elements of early treatment failure since some patients had parasitemia on day 2 but did not have another asexual parasite count until day 7, making it impossible to determine if the parasitemia on day 3 was > 25% of the baseline value or the presence of parasitemia on days 4 and 6. Furthermore, this counting scheme likely increased the reported parasite clearance time because it is impossible to determine when a patient became negative between days 2 and 7. This may also be a confounder of determining gametocyte clearance time. Late treatment failure was defined as the presence of parasitemia from day 7 to 28 regardless of symptoms.

Parasitological Measurements

Initial diagnosis was confirmed by either a positive blood smear or a positive rapid antigen test (b (1). Patients who had a positive antigen test were also required to have a positive blood smear. Inclusion criteria stated that patients were to have a parasitemia equivalent to 2% parasitemia (based on thin film). During the trial, the parasite counts were performed on Giemsa stained thin films daily until parasite clearance was reached and additionally on days 7 and 28. Upon examination of the datasets and as stated in the paragraph above, this does not seem have been implemented; some patients who were still positive at the 48 hour measurement did not have another slide examined until day 7. In order for a slide to be declared negative, a total of 200 fields per slide were examined.

Parasite density was expressed as parasitized erythrocytes per 1000 red blood cells (RBCs). The applicant also states that Giemsa stained thick films were used in determining parasite density and the data was calculated according to the formula described for Study 025. Upon examination of the datasets, it appears that some patients had parasite density reported from thin films, other patients had counts determined using thick films and some patients had parasite densities reported from both. Concordance between the estimated parasite density as determined by either thin or thick smears was noted; typically, a less than 2-fold difference was seen when both smears were performed at a single time point. Similarly, the basis for determining PCT varied among the patients; in some patients, the PCT was based on when thin films became negative and in other patients the PCT was based on when thick films became negative. This variation in the technique used to determine parasite density makes it difficult to calculate the mean PCT for all patients enrolled in the study. However, the primary efficacy endpoint of the study, the 28 day cure rate, should not be affected by this discrepancy. The applicant stated that if the blood smear was found to be positive on day 7 or if parasites reappeared following clearance, 2 mL of blood was obtained from the patient for genotyping analysis.

Genotyping

Genotyping, by PCR and restriction fragment length polymorphism (RFLP) assays, was used by the applicant to differentiate recrudescence from new infection at the (b) (4) As for the PCR studies done at the Shoklo Center, details of the assay were provided by the applicant in response to a request by the Division (letter date July 9, 2008). .

In the description of the assay described above at the Shoklo Center, differences in the size of the nested PCR product were directly compared on agarose gels. At the (b) (4), genotyping for the GLURP allele was not included for testing. The PCR primers used for MSP-1 were different; MSP-2 utilized both PCR and RFLP analysis. For MSP-2, the sequence of the primers for genotypic analysis was stated to be same as used at the Shoklo Malaria Research Unit, however, they were obtained from (b) (4) When a new batch of primers was received, a test PCR was to be performed using the three positive controls described below.

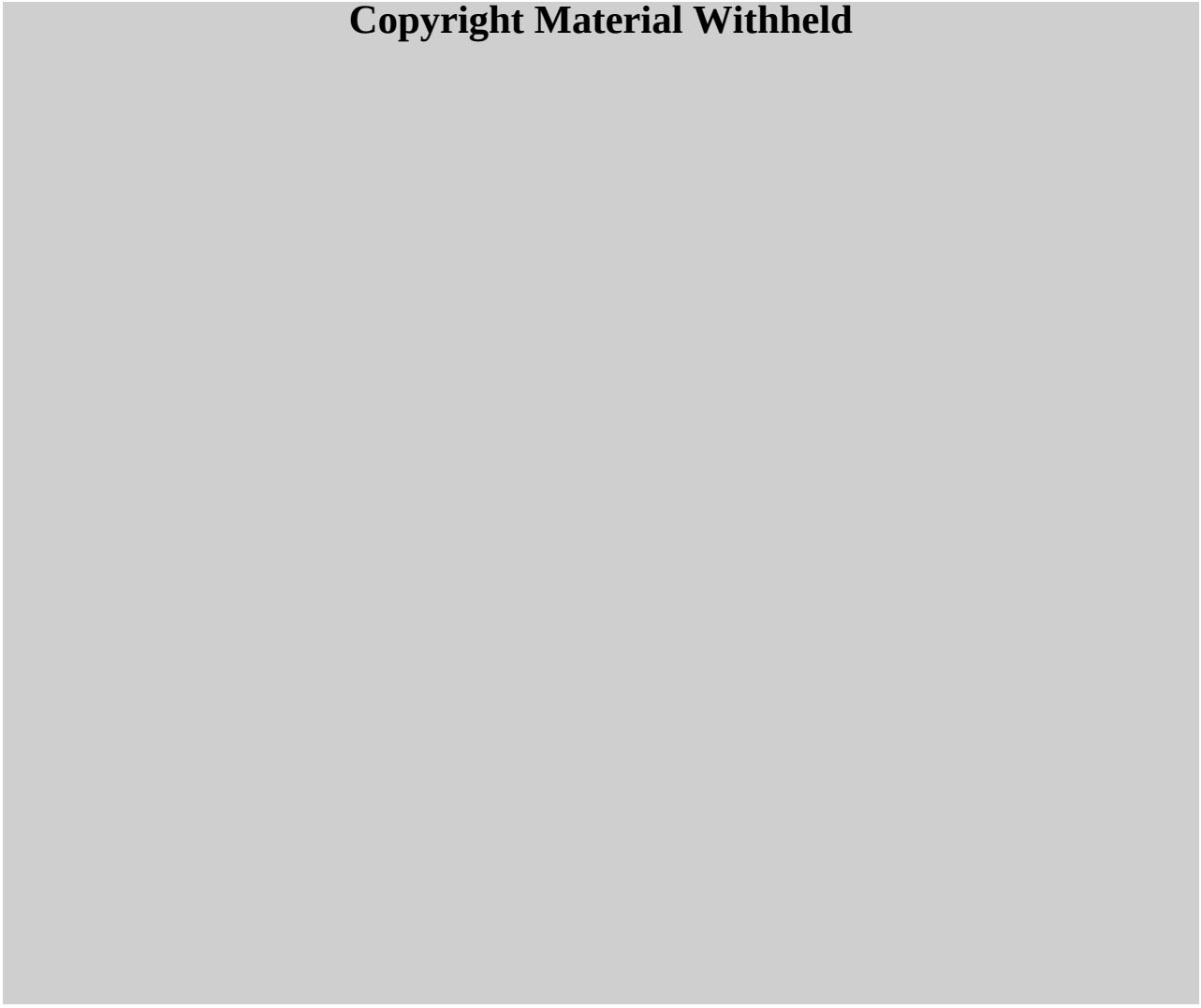
Genotyping was performed according to the method developed by Felger *et al.,*[39]. The assay involves differentiation of *P. falciparum* strains due to differences in the MSP-2 and MSP-1 alleles. The product of the nested PCR for MSP-2 was digested with the restriction enzyme HinfI and the fragments analyzed by polyacrylamide gel electrophoresis. The MSP-2 gene can be divided into allelic families: the FC27-type and 3D7-type. In single infections, two different strains can be differentiated by the nature of their FC27-type allele of MSP-2. The FC27-type alleles contain multiple copies of a 36 bp repeat in a variable region of the gene and 96 bp tandem repeats in a different region. Digestion of a FC27-type allele with *HinfI* will always yield a 137 and 115 bp product plus a third or fourth product of predictable size depending on the allele. The size of the other products will depend on the number of repeats present in the gene. A schematic of the predicted sizes of the most common FC27-type MSP-2 alleles is shown in Figure 19. Differentiation of the alleles with varying copies of:

- 36 bp repeats (IFA 46, WOS10, WOS3, WOS12 and K1) relies upon a different fragment size for the central region which is at least 162 bp in length for the IFA 46 allele and then grows by addition of 36 bp depending on the number of 36 bp repeats for the other alleles.

- 96 bp repeats (FC27/D10, WOS7, IFA 45 and WOS6) relies upon being able to discern the difference in intensity seen for the 96 bp band when run on a gel. Specifically, the 96 bp fragment will not appear if only one copy is present in the allele and a single band of 96 bp will appear if two copies are present in the allele. If three copies are present in the allele, then the intensity of staining on the gel will be twice that of an allele that has 2 copies. Relying on band intensity for differentiation of alleles is subject to error. The author of the citation notes that upon running the digested product on a gel, the intensity of the band may be difficult to discern when trying to differentiate between 3 and 4 copies of the 96 bp repeat. In this case, the undigested product can be run on a gel and the size of the product will differ depending on the number of repeats which are present in the product.

The applicant does not provide performance characteristics of the assay.

Figure 19: Predicted fragment lengths of Fc-27 type alleles following digestion of nested PCR product with *HinfI*



The citation cited by the applicant notes that the interpretation of RFLP patterns in patients with mixed infections of multiple strains of *P. falciparum* is very complex when basing the allele typing on the FC27 family alone. In cases where infections with more than one strain occur, FC27 allele-typing may be insufficient to detect multiple clones. Instead, the authors of the citation also digested MSP-2 amplification products with the restriction enzyme *DdeI*, which types the MSP-2 gene based on the 3D7 family of alleles. The applicant does not utilize this technique and thus the reliability of the assay to accurately genotype multiple infections is presumably lessened.

Three controls were included on each gel which was run for MSP-2 genotyping:

- Control 1 (Positive Control): *P. falciparum* (K1 strain) at 3 different dilutions (50/500/5000 parasitized erythrocytes per μL).
- Control 2 (Negative Control): No template added to amplification reaction
- Control 3 (Negative Control): Human blood (free of *P. falciparum* DNA)

As mentioned above, performance characteristics of the restriction digestion assay were not provided for our review. This is important because incomplete digestion of the PCR product would skew the profile, and has the potential to misidentify a genotype. A routine retesting of samples would also give confidence in the assay. The citation cited by the applicant describing this assay suggests quality control testing including retesting 10% of the samples due to the chance that infections with more than one strain or especially those with low density may not be detected. While the applicant states that negative samples were repeated, no quality control was performed on positive samples to confirm results. Moreover, a control which allowed the discernment of the different band intensities for the varying copies of the 96 bp repeat was not included.

For MSP-1 PCR, primers (listed in Table 65) were obtained from (b) (4) and were different from that used at the Shoklo Center. However, these primers were stated to identify differences in polymorphic block 2 which is the same as that for the Shoklo Center. Unlike Shoklo, which used only a single primer set to amplify the polymorphic region of MSP-1, three primer sets were used at (b) (4) Depending on the sequence of the region, a strain can be classified as belonging to one of three families (K1, MAD20 or RO33). These alleles are named for the specific strain of *P. falciparum* from which they were identified. Different numbers of repeated sequence units classify the K1 and MAD20 families, while the RO33 family contains a unique sequence not present in the other two families. The sequences of those regions which flank MAD20 and K1 alleles are conserved among the allelic variants. Three separate nested PCR reactions are performed on the primary PCR product in order to genotype the parasite. Product sizes vary from 125 – 250 bp. For MSP-1 genotyping, the positive control was performed on dilutions of the K1, MAD20 and R033 cultures (50/500/5000 parasites per μL). The negative controls for genotyping of MSP-2 were included as well.

Table 65: Primer sequences used in MSP-1 PCR genotyping at (b) (4)

The applicant stated that assays which gave positive results for either of the negative controls were discarded and repeated. There is no reporting on how often a false positive PCR was obtained which would be an important performance characteristic to have noted. It was additionally stated that an assay which gave a negative result at any dilution of the control strain was discarded and repeated up to three times. Again, the frequency of false negatives was not provided. It appears that no quality control of positive samples was included. The applicant concedes that a routine analysis of the assay's performance was not conducted.

To determine the sensitivity of the assay, parasite cultures of known density (K1 control strain) were spotted onto (b) (4) filter cards. DNA purification and the PCR for MSP-2 were performed as

for the analysis of clinical isolates. It does not appear that restriction enzyme digestion was performed and the results represent the undigested PCR products. The results, presented in Figure 20, show positive results for all dilutions of 50 parasites/μL or higher. The dilution of 25 parasites/μL did not yield a positive result whereas the sample with 10 parasites/μL yielded a product of the same size as the higher density cultures.

Another analysis to determine sensitivity of the PCR was performed as described above. This analysis also included some "blank discs" which used the same hole punch that was used to obtain the disc which was spotted with 75,000 parasites/μL. This was done to determine if cross over contamination from the hole punch presented a problem (see below for details of the assay methodology). The reaction yielded positive results for all dilutions of 25 parasites/μL or higher (Figure 21). Therefore, the sensitivity of the assay was established as 25 parasites/μL. Cross-over contamination from the hole punch did not represent a source of cross-contamination in this assay.

During the clinical trial, ten microliters of the infected blood at baseline and at reappearance of parasites was spotted onto (b) (4) DNA preparation stix (b) (4) dried and transported

to the (b) (4) for genotyping. A sample was removed from the blood collection card by using a hole punch to create a 3 mm disk. Between samples, the hole punch was used to punch 3 disks from clean filter paper to avoid cross contamination, the effectiveness of this precaution is shown in the section above. Contaminants are removed from the disk with a "purification solution" and the disk is then added directly to the PCR reaction. Genotyping of MSP-2 was performed first. The product of the primary PCR of the parasite DNA was verified on an agarose gel. This was followed by a nested PCR on the product of the initial amplification as described for the controls. The product of the nested PCR underwent *HinfI* digestion and RFLP by running the product on a 10% polyacrylamide gel. If the MSP-2 profile differed between the baseline sample and the sample obtained at treatment failure, the reappearance was classified as a new infection. If the MSP-2 profile was the same, then the samples underwent genotype analysis of MSP-1.

Parasitological Findings

Asexual Parasites

For the purpose of this review, the ITT population consisted of 162 patients, since three patients were found to not be infected with *P. falciparum* and were excluded from all efficacy analysis. The per protocol population consisted of 124 patients. Two patients (0042-00002 and 0042-00007) had parasite clearance times listed in the "eff" dataset as 0, upon examination of their parasite counts, it was found that the PCTs determined from thick films were 169.2 and 114 hours respectively. These numbers were used in this analysis of mean PCT. As stated above, patients varied in the technique used to determine parasite clearance; the PCT listed in Table 66 is a composite of the clearance times determined from thick and thin films, depending on the technique used for a particular patient. Cure rate at 14 days could not be determined since no parasitological measurements were taken near this time. Two patients experienced reappearance of parasites by day 28 (days 22 and 25) and one patient had reappearance of parasites on day 29. Since the length of time of the trial was 28 days, this patient is included among those patients with reportable recrudescence, however, this patient was considered cured for 28 day cure rate calculations. All treatment failures can be classified as RI according to WHO criteria (Table 67).

Table 66: The clearance of *P. falciparum* over time in Study 2401

Parameter		**Coartem 6 x 4**
Number of patients	**ITT**	162
	PP	124
Baseline parasitemia/µL [median (range)]	**ITT**	Thick film: 2773 (98 – 53900) Thin Film: 2.5 (0.015 – 70)*
	PP	Thick film: 2640 (98 – 53900) Thin Film: 3 (0.015 – 40)*
% patients in which parasites are cleared by 24 hours	**ITT**	24.7%
	PP	24.2%
% patients in which parasites are cleared by 48 hours	**ITT**	74.7%
	PP	74.2%
% patients in which parasites are cleared by 72 hours	**ITT**	80.2%
	PP	78.2%
% Cure rate at 7 days	**ITT**	95.1%
	PP	98.4
% Cure Rate [28 days (Uncorrected)]	**ITT**	74.1%
	PP	96.0%
% Cure Rate [28 days (Corrected)]	**ITT**	74.1%
	PP	96.0%
PCT [mean hours (range)]	**ITT**	68.5 hours (9 – 727)
	PP	63.1 hours (14 – 240.3)
Recrudescence [number of patients (Uncorrected)]	**ITT**	3
	PP	3
Recrudescence [number of patients (Corrected)]	**ITT**	3
	PP	3

*Note: The applicant states that the thin film parasite density was reported by a method other than parasitized erythrocytes per 1000 RBCs for parasite counts based on thin films

Table 67: Treatment failures as classified by the World Health Organization (based on uncorrected cure rates)*

	R-I	R-II	R-III
Coartem 6 x 4	3	0	0

*WHO definitions of treatment failure:
R-I= clearance of asexual parasitemia within 7 days, followed by recrudescence
R-II=marked reduction of asexual parasitemia but no clearance (asexual parasite counts of <25% of baseline within 48 hours after initiation of treatment but no, or only temporary clearance of asexual parasitemia within 7 days
R-III=no marked reduction of asexual parasitemia (asexual parasite counts remain >25% of baseline at 48 hours, or actually rise above baseline levels at 48 hours without clearance of asexual parasitemia within 7 days.

Genotyping Analysis
PCR was used on one of the three patients with reappearance of parasites and the patient was stated to be recrudescent. The genotype of the strain was not reported by the applicant. Additionally, the applicant reports only the uncorrected cure rates.

***P. vivax* co-infection**
At baseline, 2 patients were reported to have mixed infections of *P. falciparum* and *P. vivax*. Other than noting the presence of *P. vivax* at baseline for these patients, no enumeration was performed and the dataset shows that *P. vivax* was not present on the films examined at 24 hours. Additionally, 3 patients had appearance of *P. vivax* after baseline parasite counts were taken but before day 28 (days 12, 14 and 27). It is unknown if these patients cleared *P. vivax* parasitemia.

Gametocytes
A total of 34 patients were positive for gametocytes at some point during the trial (Table 68). Gametocyte density was not reported and the presence or absence of gametocytes was noted for each patient. At baseline, 19 patients were positive for gametocytes. The time of appearance of gametocytes for the other 15 patients ranged from to 44.2 hours (Table 69). The mean time to gametocyte clearance was 289 hours. For 4 patients who did not complete the trial, the gametocyte clearance time was calculated as day 28 minus the time of appearance of gametocytes. Due to the infrequency of blood smears, the estimation of gametocyte clearance time may be artificially high.

Table 68: Gametocyte presence during Study 2401

Number of patients with gametocytes detected on	Coartem Number of patients
Baseline	14
Day 1	26
Day 2	31
Day 3	26
Day 4	24
Day 8	12
Day 15	11
Day 29	0 (4)*
*For 4 patients who did not complete the trial, the gametocyte clearance time was calculated as day 28 minus the time of appearance of gametocytes.	

Table 69: Gametocyte clearance after treatment with Coartem

Number of patients with gametocytes	Initial gametocyte count [Mean(range)]	Gametocyte clearance time in hours [Median (range)]	Asexual parasite clearance time in hours [Median (range)]
At baseline (n=20)	Not reported	162 (24 – 670)	41 (9 – 162)
After initiation of treatment (day 1-28) (n=14)	Not reported	119 (24 – 661)	44 (9 – 163)

4.1.7. Study 2403 (Protocol CCOA566A2403)

Study 2403 was an open label, multicenter (Kilifi, Kenya; Ibadan, Nigeria; and Dar es Salaam, Tanzania) trial which determined the safety and efficacy of the 6 dose regimen of Coartem (20 mg artemether/120 mg lumefantrine over 3 days) in African infants with *P. falciparum* infection. Coartem was administered over 3 days (hours 0, 8, 24, 36, 48, and 60). Patients were followed for 28 days and the primary efficacy endpoints were the development of danger signs of severe malaria on days 1, 2 and 3, FCT, PCT, the proportion of patients with gametocytes, and the 7, 14, and 28 day cure rates. Danger signs of severe malaria are based on the 2000 WHO definition and include the inability to drink or breast-feed, vomiting everything, recent history of convulsions, lethargic or unconscious state, and inability to sit or stand up.

Patients were hospitalized during treatment and until parasite and fever clearance and then followed as outpatients with visits on days 7, 14, and 28. Patients were divided into treatment groups based on weight (5 and <10 kg, 10 and <15 kg, and 15 and ≤25 kg). Children in the 15 and ≤25 kg treatment group received a dose which was twice that of the other treatment groups (2 tablets versus 1 tablet at each time point). Additionally, another analysis, based on clinician's opinion, was performed within the 5 and <10 kg treatment group which examined the cure rates between non-immune (no previous

malaria infection is known) and semi-immune (at least one malaria infection is known or patient grew up in an endemic area and previous infections are likely) patients.

Parasitological Measurements

At screening, up to 200 thick film fields were examined to determine if *P. falciparum* asexual forms were present. If asexual forms other than *P. falciparum* were observed, 200 high power fields were examined for species other than *P. falciparum*. Patients were excluded if they had infections with other species of *Plasmodium* (*P. vivax, P. malariae,* or *P. ovale*). During the trial, a blood sample was taken by finger prick for preparation of thick smears every twelve hours (i.e. before each dose) starting with the 3rd dose (24 hours) and extending until the last dose (hour 60), and then on days 7, 14, and 28. Examination of the parasitological dataset revealed that parasite counts were also performed between hours 5 and 8 for most of the patients. In order for a slide to be declared negative, a total of 200 fields were examined. If infection reemerged, daily parasite counts were reinstated. Parasite counts were based on counting the number of parasitized erythrocytes per 200 WBCs. If this number was less than 10 parasites, then counting was extended to 500 WBCs. Parasite density was calculated according to the formula described for Study 025.

If gametocytes were observed, a gametocyte count was performed. No further details were given concerning how gametocytes were enumerated.

The applicant stated that the blood films were independently reviewed by the (b) (4) for verification of the presence or absence of asexual parasites and gametocytes. The quality control was based on a total of 331 smears (out of 3067 total smears). Additionally, 334 smears were reviewed in a similar fashion for the presence or absence of gametocytes. Results for the review of asexual parasite presence are shown in Table 70. If the peer review data is taken as the true result, then the analysis by the trial investigators had both high specificity and high sensitivity and was within acceptable limits.

Table 70: Results of peer review analysis for presence of asexual parasites

	Peer Review Conclusion		
Applicant Conclusion	**Positive (n)**	**Negative (n)**	**Total (n)**
Positive	106	25	131
Negative	7	193	200
Total	113	218	331

The peer review results, however, show that the assay to identify gametocytes performs well in correctly identifying patients who are negative for gametocytes (310 of 326 were concordant); however, the assay is unable to adequately identify those patients who are positive for gametocytes (3 of 8 were concordant, see Table 71). This aspect may be of particular importance since gametocyte clearance was a secondary endpoint of the studies and the inability to correctly identify patients with gametocytes may falsely classify a patient as negative due to a lack of sensitivity. The applicant states that the original data obtained at the study center was used in the parasitological analysis.

Table 71: Results of peer review analysis for presence of gametocytes

	Peer Review Conclusion		
Applicant Conclusion	**Positive (n)**	**Negative (n)**	**Total (n)**
Positive	3	5	8
Negative	16	310	326
Total	19	315	334

Genotyping
Though only uncorrected cure rates are included in the study report, PCR was utilized to distinguish reinfection from recrudescence upon reappearance of parasites in 34 patients. All steps of the PCR assay were performed at (b) (4) laboratory and are the same as discussed above for Study 2401.

Parasitological Findings
Asexual Parasites
Patients were to be enrolled if their baseline parasitemia was between 1000 and 100,000 parasites per µL. However, 3 patients had baseline counts of > 100,000 parasites per µL and were included in efficacy analysis. The actual baseline parasitemia range was 1000 to 137,760 parasites/µL with a median of 18,488/µL. The intent to treat population (n=309) consisted of 153 patients in the 5 to <10 kg group, 110 patients in the 10 to <15 kg group, and 46 patients in the 15 to ≤25 kg group. One patient in the 5 to <10 kg group (0002-00147) only had a baseline parasite measurement taken, the applicant lists this patient's PCT as Hour 0. For the purpose of this review this patient was excluded from analysis. The per protocol population consisted of 300 patients (149 patients in the 5 to <10 kg group, 107 patients in the 10 to <15 kg group, and 44 patients in the 15 to ≤25 kg group). The datasets provided by the applicant do not distinguish those patients in the 5 to <10 kg treatment group who were classified as being partially immune or non-immune, therefore an independent analysis of the study results between these groups is not possible. The results in Table 72 show that cure rates at 7, 14, and 28 days as well as parasite clearance time were similar across groups. When comparing PCT between non-immune and semi-immune patients in the 5 to <10 kg treatment group, semi-immune patients had a higher overall cure rate when compared to non-immune patients (88.6% versus 82.5%, respectively). A total of 34 patients had reappearance of parasites after microscopically confirmed clearance, parasites reappeared between day 24 and 28, with the median day of reappearance of day 27. All treatment failures can be classified as RI according to WHO criteria (Table 73).

Genotyping Analysis
Of the 34 patients who experienced reappearance of parasites based on positive blood smears, 11 were reported as recrudescent and 23 as new infections by PCR genotyping (Table 74). The observation that many more patients were subject to new infections rather than recrudescence is opposite of what is seen for most of the other studies. This may be due to either the patient population or the geographical location of this study. The actual gel results for this study could not be located by the applicant and were not provided for our review. As discussed for Study 2401, the results of PCR should not be used for evaluating the efficacy of the drug.

Table 72: The clearance of *P. falciparum* over time in Study 2403

Parameter		5 - <10 kg	10 - <15 kg	15 - ≤25 kg
Number of Patients	**ITT** (n=309)	153	110	46
	PP (n=300)	149	107	44
Baseline parasitemia/µL [median (range)]	**ITT**	17581 (1080 – 100000)	20929 (1373 – 137760)	14726 (1000 – 104919)
	PP	18400 (1080 – 100000)	21758 (1373 – 137760)	13323 (1000 – 104919)
% parasite reduction at 24 hours median(range)	**ITT**	100 (-42.1 – 100) n=150	99.9 (-695.5 – 100)	100 (48.5 – 100) n=45
	PP	100 (-42.1 – 100) n=147	99.9 (-695.5 – 100)	100 (48.5 – 100) n=45
% patients in which parasites are cleared by 24 hours	**ITT**	57.3 n=150	48.2	68.9 n=45
	PP	56.5 n=147	47.7	68.2
% patients in which parasites are cleared by 48 hours	**ITT**	97.4 n=152	99.1	97.8
	PP	98	99.1	97.7
% patients in which parasites are cleared by 72 hours	**ITT**	100	100	100
	PP	100	100	100
% Cure rate at 7 days	**ITT**	100	100	100
	PP	100	100	100
% Cure rate at 14 days	**ITT**	100	100	100
	PP	100	100	100
% Cure Rate [28 days (Uncorrected)]	**ITT**	86.9	85.5	89.1
	PP	89.2	87.9	90.9
% Cure Rate [28 days (Corrected)]	**ITT**	94.8	93.6	93.5
	PP	97.3	96.3	95.5
PCT [mean hours (range)]	**ITT**	30.2 (5.3 – 68)	31.1 (7.7 – 64)	26.7 (7.2 – 71.1)
	PP	30.5 (5.3 – 68)	31 (7.8 – 59.9)	26.3 (7.2 – 71.1)
Recrudescence [number of patients (Uncorrected)]	**ITT**	17	13	4
	PP	16	13	4
Recrudescence [number of patients (Corrected)]	**ITT**	5	4	2
	PP	4	4	2

Note: Unless otherwise noted, the (n) for each analysis is listed in the first two rows of the table; some subjects did not have measurements taken for a certain parameter and thus were excluded from those analyses.

Table 73: Treatment failures as classified by the World Health Organization (based on uncorrected cure rates)*

	R-I	R-II	R-III
5 - <10 kg	17	0	0
10 - <15 kg	13	0	0
15 - ≤25 kg	4	0	0
Total	34	0	0

*WHO definitions of treatment failure:
R-I= clearance of asexual parasitemia within 7 days, followed by recrudescence
R-II=marked reduction of asexual parasitemia but no clearance (asexual parasite counts of <25% of baseline within 48 hours after initiation of treatment but no, or only temporary clearance of asexual parasitemia within 7 days
R-III=no marked reduction of asexual parasitemia (asexual parasite counts remain >25% of baseline at 48 hours, or actually rise above baseline levels at 48 hours without clearance of asexual parasitemia within 7 days.

Table 74: Results of PCR genotype analysis on paired patient blood samples

Recrudescence vs. New infection	**5 - <10 kg**	**10 - <15 kg**	**15 - ≤25 kg**
New infection	12	9	4
Recrudescence	5	4	2
PCR not done	0	0	0
Total	17	13	4

***P. vivax* co-infection**
Patients who were found to be co-infected with *P. vivax* were excluded from the trial.

Gametocytes
Of the 11 patients who presented with gametocytes at baseline, 6 were in the 5 to <10 kg group, 5 in the 10 to <15 kg and no patients were in the 15 to ≤25 kg group (Table 75). An additional 15 patients had emergence of gametocytes after baseline (days 1 – 2) during the trial (7 in 5 to <10 kg group, 6 in the 10 to <15 kg group and 2 in the 15 to ≤25 kg group). The median of gametocyte density at baseline was 77 gametocytes per μL (range: 32 to 254) (Table 76). The median time to gametocyte clearance was 50.4 hours (range: 7.8 to 307.7 hours). The lack of emergence of gametocytes after day 2 is probably more reflective of elimination of the asexual stage of the parasite due to drug treatment.

Table 75: Gametocyte presence during Study 2403

Time of detection of gametocytes	Number of patients		
	5 - <10 kg	10 - <15 kg	15 - ≤25 kg
Baseline	6	5	0
Day 1	9	9	2
Day 2	8	6	1
Day 3	5	2	0
Day 4	2	2	0
Day 8	2	0	0
Day 15	0	0	0
Day 29	0	0	0

Table 76: Gametocyte data for Study 2403

Group	Time of gametocyte appearance	Initial gametocyte count [Mean (range)]	Gametocyte clearance time in hours [Median (range)]	Asexual parasite clearance time in hours [Median (range)]
5 to <10 kg	Patients with gametocytes at baseline (n=6)	163 (16 – 254)	23.95 (7.8 – 67.5)	35.8 (23.8 – 59.8)
	Patients with gametocytes after initiation of treatment (day 1-28) (n=7)	98 (16 – 194)	27.9 (12 – 307.7)	35.9 (23.8 – 59.9)
10 to <15 kg	Patients with gametocytes at baseline (n=5)	85 (57 – 128)	23.1 (7.9 – 23.8)	23.8 (8 – 35.8)
	Patients with gametocytes after initiation of treatment (day 1-28) (n=6)	71 (25 – 207)	12 (12 – 138.1)	35.9 (24 – 59.9)
15 to ≤25 kg	Patients with gametocytes at baseline (n=0)	NA	NA	NA
	Patients with gametocytes after initiation of treatment (day 1-28) (n=2)	38 (38 ,39)	14 (12 ,16)	15.9 (7.9, 23.9)

4.1.8. Study 2303 (Protocol COA566B2303)

Study 2303 was a randomized, investigator blinded, multicenter, parallel group study which explored the efficacy, safety and tolerability of a dispersible versus crushed tablet formulation of Coartem in the treatment of acute, uncomplicated *P. falciparum* malaria in infants and children. The dosage forms were dissolved in 10 mL of water for administration. The patient population was defined as children under 12 years of age who weighed ≥ 5 kg to < 35 kg. These patients were divided into 3 groups based on body weight as Group 1: ≥ 5 to <15 kg, Group 2 ≥15 to <25 kg, and Group 3 ≥25 to <35 kg. For the purpose of this review, all efficacy data is compared on the basis of the dosage form and not weight class. The study took place at 8 study centers under different investigators:

- Site 101: Kilifi, Kenya
- Site 102: Kisumu, Kenya
- Site 103: Kisumu, Kenya (same area as site 102)
- Site 201: Maputo, Mozambique
- Site 301: Bagamoyo, Tanzania
- Site 302: Zanzibar, Tanzania
- Site 601: Bamako, Mali
- Site 801: Cotonou, Republic of Benin

Coartem was administered as six doses over 3 days (hours 0, 8, 24, 36, 48, and 60). If possible, patients were hospitalized through day 7 and then followed until day 42. The primary efficacy endpoint was the 28 day PCR corrected cure rate. Secondary efficacy variables included the 7 day cure rate, 14 day PCR corrected cure rate, PCT, FCT and time to gametocyte clearance. Exploratory variables included the development of danger signs of severe malaria (same as defined above for study 2403), PCR corrected 42 day cure rate, early treatment failure, late clinical failure or late parasitological failure and exploration of an adequate clinical and parasitological response. Early treatment failure was defined as being diagnosed with one of the following:

- Development of danger signs of malaria on day 1, 2 or 3 in the presence of parasitemia,
- Parasitemia on day 2 higher than day 0 count irrespective of the axillary temperature,
- Parasitemia on day 3 with axillary temperature ≥ 37.5°C, or
- Parasitemia on day 3 equal or more than 25% of the count on day 0.

Late clinical failure was defined as being diagnosed with one of the following:

- Development of danger signs or severe malaria after day 3 in the presence of parasitemia without previously meeting any of the criteria of early treatment failure, or
- Presence of parasitemia and axillary temperature ≥ 37.5°C from day 4 to day 14 without previously meeting any criteria of early treatment failure.

Late parasitological failure was defined as the presence of parasitemia between day 14 and day 42 and axillary temperature < 37.5°C without meeting any of the previously mentioned criteria for early treatment failure. The adequate clinical and parasitological response was defined as the absence of

parasitemia from days 28 to 42 irrespective of axillary temperature and without meeting any of the criteria defined for early treatment failure.

Parasitological Measurements
At screening, up to 200 thick film fields were examined to determine if *P. falciparum* asexual forms were present. If asexual forms were observed, 200 high power fields were examined for species other than *P. falciparum*. Counting of *P. falciparum* asexual parasites was based on counting the number of parasitized erythrocytes per 200 WBCs. If this number was less than 10 parasites, then counting was extended to 500 WBCs. Parasite density was calculated according to the formula described for Study 025. After treatment commenced, a blood sample was taken for determination of parasite density at hours 8, 24, 36, 48 and 60 as well as on days 4, 7, 14, 28 and 42. In order for a slide to be declared negative, a total of 200 fields were examined. If infection reemerged, daily parasite counts were reinstated. If gametocytes were seen during examination of slides, a gametocyte count was performed per 1000 WBCs.

At some sites (102, 103 and 301), two microscopists read the slides and the parasite density was calculated as the average of these two counts. If results between the two microscopists were discordant, then a third microscopist performed a count. In this case, the parasite density was calculated as the mean of the two most concordant results. At the other sites (101, 201, 302, 601, and 801), a random selection of 10% of the patients was selected for quality control analysis. A microscopist (preferably not the one who performed the initial count) was blinded to the identity of the slide and performed an evaluation of the slide and rated it as a positive or negative. The results of the reanalysis were compared to the original conclusions and if a more than 10% of the slides were discordant, a review of all study results would take place. The results of the controls by site were as follows:

- Site 101: 10% of the slides were rechecked by a third microscopist; of these, 5/135 deviated by greater than 50% from the original count
- Site 102: All slides were double checked. The applicant does not report any discrepancies.
- Site 103: The applicant does not report any discrepancies.
- Site 201: 115 slides (~10%) were rechecked and 9 (7.8%) were found to be discordant. Six of those 9 slides which were found to be positive had low parasite counts (< 5 per μL).
- Site 301: The applicant does not report any discrepancies.
- Site 302: Approximately 83% of the slides were rechecked and discrepancy was found in 14 smears (5.8%). These slides were subjected to a third reading and the two results which matched were reported. Most of the slides which were discordant had low parasite densities.
- Site 601: 5 of 216 slides (2.3%) were found to be discordant.
- Site 801: Of 137 slides randomly selected and rechecked, 2 were found to be discordant (1.5%).

Genotyping
PCR and RFLP assays were utilized to distinguish reinfection from recrudescence upon reappearance of parasites in 186 patients. All steps of the PCR assay were performed at (b) (4) and are the same as discussed above for Study 2401. The applicant stated that 10% of all blood samples were reanalyzed for quality control.

Parasitological Findings

Asexual Parasites

Patients were to be enrolled if their baseline parasitemia was ≥ 2000 and < 200,000 parasites per µL. However, 4 patients had baseline counts of < 2000 parasites per µL and 2 patients had counts > 200,000 parasites per µL. These patients were included in efficacy analysis. The baseline parasitemia ranged between 520 and 628,571 parasites/µL with a median of 29,200 parasites/µL. The intent to treat population (n=886) consisted of 444 patients in the crushed tablet group, and 442 patients in the dispersible tablet group. The per protocol population consisted of 804 patients (406 patients in the crushed tablet group, and 398 patients in the dispersible tablet group). Two patients from each treatment group were regarded as early treatment failures due to higher parasitemia on day 2 compared to the baseline value (Table 77). Comparisons between the different dosage forms show that cure rates at 7, 14, and 28 days as well as parasite clearance time were similar (Table 78). A total of 186 patients had reappearance of parasites after microscopically confirmed clearance, parasites reappeared between days 8 and 44, with the median day of reappearance on day 34. All treatment failures can be classified as RI according to WHO criteria (Table 79).

Table 77: Early treatment failures based on parasitological data

Dosage Form	Parasitemia on day 2 higher than baseline	Parasitemia on day 3 and temperature > 37.5°C	Parasitemia on day 3 with axillary temperature ≥ 37.5°C	Parasitemia on day 3 ≥ 25% of baseline count
Crushed Tablet	2	0	0	0
Dispersible Tablet	2	0	0	0

Twenty six patients were identified that have their PCT data listed incorrectly. In the datasets, these patients are listed as "censored" and are included in the ITT analysis but not the PP analysis performed by the applicant. The PCT listed for these patients is artificially low; some patients had no reported parasite density counts performed and their PCT is listed as 0, while others discontinued the trial; however, the PCT for these patients was listed as the hour at which the last smear was performed (which was still positive). For the purpose of this review, all these patients were excluded from analyses and the ITT population consists of 860 patients instead of 886.

Table 78: The clearance of *P. falciparum* over time in Study 2303

Parameter		Crushed tablet	Dispersible Tablet
Number of Patients	**ITT** (n=860)	433	427
	PP (n=804)	406	398
Baseline parasitemia/µL [median (range)]	**ITT**	32312 (1581 – 628571)	25936 (520 – 196840)
	PP	32276 (2018 – 194955)	25760 (2039 – 196840)
% parasite reduction at 24 hours [median(range)]*	**ITT**	100 (-4865 – 100) n=403	100 (-417 – 100) n=398
	PP	100 (-4865 – 100) n=369	100 (-417 – 100) n=366
% patients in which parasites are cleared by 24 hours *	**ITT**	47.1	49.6
	PP	38.9	40.7
% patients in which parasites are cleared by 48 hours	**ITT**	93.5	92.5
	PP	93.6	93.0
% patients in which parasites are cleared by 72 hours	**ITT**	99.3	99.3
	PP	99.3	99.2
% Cure rate at 7 days	**ITT**	100	99.5
	PP	100	100
% Cure rate at 14 days	**ITT**	99.5	99.5
	PP	99.8	100
% Cure Rate [28 days (Uncorrected)]	**ITT**	86.4	87.8
	PP	90.4	92.5
% Cure Rate [28 days (Corrected)]	**ITT**	95.8	95.3
	PP	98	97.2
PCT [mean hours (range)]	**ITT**	32 (6.6 – 165.6)	31.7 (6.5 – 169)
	PP	31.9 (6.6 – 165.6)	31.5 (6.5 – 169)
Recrudescence [number of patients (Uncorrected)]	**ITT**	47	38
	PP	37	25
Recrudescence [number of patients (Corrected)]	**ITT**	6	6
	PP	6	6

Note: Unless otherwise noted, the (n) for each analysis is listed in the first two rows of the table; some subjects did not have measurements taken for a certain parameter and thus were excluded from those analyses.
*Parasite reduction and clearance at 24 hours used counts from hours 20 – 28 since not all patients had 24 hour counts.

Table 79: Treatment failures as classified by the World Health Organization (based on uncorrected cure rates)*

Dosage Form	R-I	R-II	R-III
Crushed tablet	47	0	0
Dispersible tablet	38	0	0
Total	85	0	0

*WHO definitions of treatment failure:
R-I= clearance of asexual parasitemia within 7 days, followed by recrudescence
R-II=marked reduction of asexual parasitemia but no clearance (asexual parasite counts of <25% of baseline within 48 hours after initiation of treatment but no, or only temporary clearance of asexual parasitemia within 7 days
R-III=no marked reduction of asexual parasitemia (asexual parasite counts remain >25% of baseline at 48 hours, or actually rise above baseline levels at 48 hours without clearance of asexual parasitemia within 7 days.

Genotyping Analysis

Of the 186 patients who experienced reappearance of parasites based on positive blood smears, the median day of reappearance was day 34 (range: day 8 to 44). Eighty five of the reappearances occurred before day 29 and were included in the efficacy analysis (Table 80). Results of genotyping for these isolates showed that 67 were new infections, 12 recrudescences, and 6 were considered as unclear, missing or not performed. The genotype found for each individual patient was not included in the study report. Gel results for both MSP-1 and MSP-2 genotyping were provided and were generally of acceptable quality. The observation that many more patients were subject to new infections rather than recrudescence is opposite of what is seen for the studies in Thailand and China. This may be due to either the patient population or high transmission in the geographical location of this study.

The applicant stated that the PCR on 10% of the blood samples would be repeated for quality control, however, no results of this analysis were included in the study report. As discussed for Study 2401, the efficacy should be evaluated based on blood smear results and not genotyping. This may be due to either the patient population or high transmission in the geographical location of this study.

The applicant stated that the PCR on 10% of the blood samples would be repeated for quality control, however, no results of this analysis were included in the study report. As discussed for Study 2401, the efficacy should be evaluated based on blood smear results and not genotyping.

Table 80: Results of PCR genotype analysis on paired patient blood samples (on reappearances occurring before day 29)

Recrudescence vs. New infection	Crushed tablet	Dispersible tablet
New infection	38	29
Recrudescence	6	6
PCR not done, results missing or unclear	3	3
Total	47	38

***P. vivax* co-infection**

Though there were no exclusion criteria for patients with mixed infections of *P. falciparum* and other species of *Plasmodia*, there were no cases of mixed infections reported in this study.

Gametocytes

Of 40 patients who presented with *P. falciparum* gametocytes at baseline, 21 and 19 were in the crushed and dispersible tablet groups, respectively (Table 81). An additional 67 patients had emergence of gametocytes after baseline (days 1 to 4) during the trial. The median of gametocyte density at baseline was 39 gametocytes per µL (range: 5 to 11,816). The median time to gametocyte clearance was 27.7 hours (range: 4.2 to 660 hours; Table 82). The lack of emergence of gametocytes after day 4 is probably more reflective of elimination of the asexual stage of the parasite after drug treatment.

Table 81: Gametocyte presence during Study 2303

Time of detection of gametocytes	Number of patients	
	Crushed tablet	Dispersible tablet
Baseline	21	19
Day 1	40	33
Day 2	42	36
Day 3	34	27
Day 4	14	13
Day 8	13	11
Day 15	6	3
Day 29	1	1

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Table 82: Gametocyte data for Study 2303

Group	Time of gametocyte appearance	Initial gametocyte count [Mean(range)]	Gametocyte clearance time in hours [Median (range)]	Parasite clearance time in hours [Median (range)]
Crushed tablets	At baseline (n=21)	50.5 (8-200)	61.1	35.5 (23 – 47.5)
	After initiation of treatment (day 1-28) (n=34)	111.6 (5- 1360)	16.6	35.9 (7.8 – 165.6)
Dispersible tablets	At baseline (n=19)	712.4 (5 – 11816)	36.4 (7.4 – 164.6)	33.8 (23.7 – 66.2)
	After initiation of treatment (day 1-28) (n=33)	79.9 (1 – 1039)	19.8 (1.3 – 660.7)	35.7 (8 – 63.8)

4.1.9. Other Studies

Sixteen study reports were provided but datasets were not available for review. These studies are summarized briefly (Table 83). Twelve of the 16 clinical studies were a 4-dose regimen studies and did not achieve high cure rates. All of the studies except 1 were conducted at centers outside of China. In China, the 4 dose regimen of Coartem was able to achieve a cure rate which exceeded 90% in both the ITT and PP (Study AB/MO2).

There were 3 studies supporting the 6-dose regimen of Coartem. The 28 day uncorrected cure rates (>90 %) were available for two (Studies 1030 and bd01) of the three studies conducted in Vietnam and Bangladesh and support efficacy of Coartem in the treatment of uncomplicated falciparum malaria.

Seven of theses studies used PCR to distinguish recrudescence from reinfection. PCR was conducted at the Shoklo Malaria Research Unit (Studies 1008 and 2412), at the (b) (4) (Study 1011), at the (b) (4) (Studies 1003 and 1007), and (b) (4) (Studies 1009 and 1010). Here again the performance characteristics of the assay were not included for review. There are several limitations of the assay and results must be interpreted with caution. Some of these limitations were also discussed by the applicant in the NDA submission in one of the study reports (Study 1003; for details *see Appendix*).

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Table 83: Summary of 16 other clinical studies

Study (location)	Age of patients (years)	Study Design	Diagnostic procedure	Treatment arms	Median PCT in ITT (hours) (range)	Median percent parasite reduction at 24 hours (range)	28 day cure rate		PCR (lab)	PCR results (new infections/total reappearances tested)
							ITT	PP		
1003 (Thailand) n=219	5 - 12	R, OL	Thick blood smears	Coartem: 4 doses (48hrs)	40 (16 – 89)	98.6 (increase – 100)	43.2	60.8	Yes (b) (4)	10/28
				Quinine: (10 mg q8h days 1 – 4, 15 mg q8h days 4 – 8)	77 (17 – 161)	67.3 (increase – 100)	47.2	71.8		4/20
1004 (Thailand) n=252	≥ 12	R, DB	Thick blood smears	Coartem: 4 doses (48hrs)	43 (11 – 89)	98.6 (increase – 100)	62.7	69.3	ND	NA
				Mefloquine: 750 and 800 mg at time 0 and 8 hours, respectively	66 (15 – 160)	76.1 (increase – 100)	77.8	82.4		NA
1008 (Thailand) n=617	≥ 5	R, OL	Thick blood smears	Coartem: 4 doses (48hrs)	ND	100 (increase – 100)	73.1	82.1	Yes (Shoklo Malaria Research Centre, see Study 025 for details)	9/48
				MAS: Artesunate (4mg/kg days 1 – 3) and Mefloquine (15 mg/kg on day 2 and 10 mg/kg on day 3)	ND	100 (increase – 100)	84.1	97.3		1/7
2412 (Thailand) n=87	≥ 12	R, OL	Thick blood smears	Coartem: 6 doses (60hrs)	ND	85.4	ND	ND	Yes (Shoklo Malaria Research Centre, see Study 025 for details)	Corrected Cure rate: 91.5%
				Atovaquone/proguanil sid days 1 - 3	ND	100	ND	ND		Corrected Cure rate: 100
				Artesunate 4mg/kg/day Days 1 – 3 and mefloquine 15 mg/kg Days 2 - 3	ND	93.8	ND	ND		Corrected Cure rate: 93.8
1030 (Vietnam) n=83	15 - 55	R, OL	Thick films for parasite density, thin films for clearance confirmation	Coartem: 6 doses (60hrs)	Mean: 43.2 (ND)	99.98 (ND)	97.8	97.2	ND	NA
				MAS: Artesunate (4mg/kg days 1 – 3) and Mefloquine (15 mg/kg on day 2 and 10 mg/kg on day 3)	Mean: 40.8 (ND)	99.99 (ND)	100	100		NA
AB/MO1 (China) n=102	13 – 59	OL	Thick blood smears	Coartem: 4 doses (48)	30 (24 – 54)	99.4 (14.9 – 100)	96.1	ND	ND	NA

Table 83 (continued): Summary of 16 other clinical studies

Study (Location)	Age of patients (years)	Study Design	Diagnostic procedure	Treatment arms	Median PCT in ITT (hours) (range)	Median percent parasite reduction at 24 hours (range)	28 day cure rate		PCR (lab)	PCR results (new infections/total reappearances tested)
							ITT	PP		
bd01 (Bangladesh) n=206	≥12	R, DB	Thick blood smears	Coartem: 6 doses (64hrs)	ND	ND	90.3	93	ND	NA
				Quinine (10 mg q8h days 1 - 3); Fansidar (500 mg/ kg sulphadoxine and 25mg/kg pyrimethamine at hour 64)	ND	ND	87.4	88.2		NA
1007 (India) n=189	≥16	R, DB	Thick blood smears	Coartem: 4 doses (48hrs)	36 (6 – 95)	98.8 (43.3 – 100)	69.7	95.4	Yes (b) (4)	0/3
				Chloroquine (600 mg at hour 0, then 300 mg at hours 6, 24, 28)	60 (17 – 167)	70.7 (increase – 100%)	16.7	19.7		2/49
ic04 (Senegal) n=72	≥18	R, OL	Thick blood smears	Coartem: 4 doses (48hrs)	24 (ND)	94.3 (ND)	day 14: 100%	day 14: 100%	ND	NA
				Chloroquine: 600 mg at hours 0, 6, 24, and 30, then 300 mg at hour 48.	48 (ND)	54.7 (ND)	day 14: 63.9%	day 14: 76.7%		NA
ic04 (Cameroon) n=60	≥18	R, OL	Thick blood smears	Coartem: 4 doses (48hrs)	48 hours (ND)	76.8 (ND)	day 14: 93.3%	day 14: 93.3%	ND	NA
				Fansidar: (dosage not given) at hour 0	168 hours (ND)	49.2 (ND)	day 14: 53.3%	day 14: 53.3%		NA
1011 (Tanzania) n=260	1 – 5	R, OL	Thick blood smears	Coartem: 4 doses (48)	ND	97.8 (ND)	ND	63.6	Yes (b) (4) laboratory, see Study 2401 for details)	14/37
				Chloroquine (10 mg/kg at hour 0, then 5 mg/kg at hours 6, 24 and 48)	ND	59 (ND)	ND	5		7/137

Table 83 (continued): Summary of 16 other clinical studies

Study (location)	Age of patients (years)	Study Design	Diagnostic procedure	Treatment arms	Median PCT in ITT (hours) (range)	Median percent parasite reduction at 24 hours (range)	28 day cure rate		PCR (lab)	PCR results (new infections/total reappearances tested)
							ITT	PP		
1010 (The Gambia) n=287	1 – 5	R, DB	Thick blood smears	Coartem: 4 doses (48hrs)	ND	94.9 (ND)	day 14: 77.1	day 14: 93.3	Yes (b) (4)	6/8 Corrected cure rate (PP): 98.3
				Fansidar (hours 0, 8, 12 and 48)	ND	62.4 (ND)	day 14: 87.4	day 14: 97.7		0
1009 (The Gambia) n=60	5 – 14	OL, NC	Thick blood smears	Coartem: 4 doses (48hrs)	36 (12 – 60)	99.3 (51.2 - 100)	71.7	70.9	Yes (b) (4)	12/14 Corrected cure rate (PP): 92.7%
br01 (Brazil) n=59	≥16	R, OL		Coartem: 4 doses (48)	48 (ND)	ND	day 5 100 %	day 5 100 %	ND	NA
				Quinine: 1500 mg q8h days 1 -3; and doxicicline 1100 mg q12h days 1 - 5	72 (ND)	ND	day 5 100 %	day 5 100 %		NA
1014 (Europe) n=103	≥ 18	R, DB	Thick blood smears	Coartem: 4 doses (48)	32 (4 – 72)	99.7 (25.9 – 100) (PP population)	76.5	82.2	ND	NA
				Halofantrine (500 mg, given at 0, 6, and 12 hours on both day 1 and 8)	48 (18 – 163)	89.6 (increase – 100) (PP population)	78.8	100		NA
1005 (Europe)	23 (≥ 18)	R, OL	Thick blood smears	Coartem: 4 doses (48)	38 (22 – 88)	99.2 (47.9 – 100)	58.3	100	ND	NA
				Quinine (600 mg tid until PCT reached); then 3 tablets Fansidar (total: 75 mg pyrimethamine, 1500 mg sulfadoxine)	69 (34 – 133)	87.6 (increase – 98.4)	72.7	100		NA

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4.2 Interpretative criteria

The applicant has not proposed any interpretive criteria and breakpoints.

5. DISCUSSION

The applicant has requested approval of Coartem for the treatment of uncomplicated *P. falciparum* malaria. Coartem is a fixed dose therapy combination of artemether and lumefantrine in a 1:6 ratio.

Nonclinical Microbiology:

Mechanism of action

Artemether is rapidly metabolized to dihydroartemisinin (DHA). Chemically, both artemether and DHA are sesquiterpene lactones containing an endoperoxide bridge. DHA and artemether were shown to accumulate selectively in erythrocytes infected with *P. falciparum*. Erythrocytes infected with ring, trophozoite and schizont forms had greater than 300 fold uptake of the drug whereas uninfected erythrocytes had less than 2-fold uptake of the drug (Figure 2). The study showed that the uptake process is reversible and saturable. Competition studies indicated that the mechanism by which DHA accumulates in infected erythrocytes is the same as that for artemether. The anti-malarial activity of artemether and DHA has been attributed to endoperoxide. The activity of artemether is enhanced in the presence of high oxygen tension and other free-radical generating compounds such as riboflavin. Anti-oxidants (free radical scavengers) such as vitamin E block the anti-malarial activity of artemether. Artemether was shown to covalently bind to proteins including hemoglobin, in addition, available studies showed that artemether inhibits nucleic acid and protein synthesis.

Lumefantrine has an aryl-amino alcohol group structure. The exact mechanism by which lumefantrine exerts its anti-malarial effect is not well defined. Available data suggest lumefantrine inhibits nucleic acid and protein synthesis but not glycolysis. Lumefantrine was shown to inhibit the formation of β-hematin by forming a complex with hemin.

Activity *in vitro*

The activity of artemether, DHA, and lumefantrine was measured against the erythrocytic stages of laboratory strains and clinical isolates from Thailand, Africa, China, Philippines and French Guiana as measured by incorporation of ^{3}H–hypoxanthine or by microscopic method. The results, expressed as 50% inhibitory concentration (IC_{50}) in Table 84 show that artemether and DHA IC_{50} values against the laboratory strains and clinical isolates were < 23 ng/mL. The IC_{50} values for artemether and DHA were similar in activity against isolates with low and high chloroquine IC_{50} values.

Studies show that lumefantrine IC_{50} values against *P. falciparum* laboratory or clinical isolates ranged from 3.3 – 126.9 µg/mL. A study in South Africa, showed that lumefantrine IC_{50} values were somewhat lower against isolates with high chloroquine IC_{50} value compared to those with lower chloroquine IC_{50} values, suggesting variable sensitivity to lumefantrine in these regions. In the absence of interpretive criteria/breakpoints, the clinical relevance of the IC_{50} values is unknown.

Table 84: In vitro activity of artemether, DHA, and lumefantrine against erythrocytic stages of *Plasmodium falciparum*

	Lumefantrine	**Artemether**	**Dihydroartemisinin**
• Against laboratory strains			
IC_{50} ng/mL [range, (n)]			
Microscopic method	1.01 - 361.93 (n = 8)	0.05- 1.82 (n = 8)	0.1 - 6.54 (n = 7)
Hypoxanthine incorporation	ND	0.40 - 6.77 (n = 6)	0.25 - 1.48 (n = 3)
IC_{90} ng/mL [range, (n)]			
Microscopic method	50.57 - 240.8 (n = 3)	0.35 -10.6 (n = 4)	ND
• Against clinical isolates			
IC_{50} ng/mL [range, (n)]			
Microscopic method	3.30 – 12.69 (n = 384)	0.06 - 18.91 (n = 31)	ND
Hypoxanthine incorporation	ND	0.07 - 22.69 (n = 1052)	0.15 - 6.6 (n = 128)
IC_{90} ng/mL [range, (n)]			
Microscopic method	2.0 – 126.9 (n = 137)	8.74 (n = 31)	ND

Note: IC_{50} = 50% Inhibitory Concentration; IC_{90} = 90% Inhibitory Concentration;
n = number of laboratory or clinical strains tested; ND = not determined;

The combination of artemether with lumefantrine (ratios between 10:1 and 1:100) *in vitro* was shown to be 3 to 100 fold more active than either drug alone. No antagonism was reported.

No studies were performed to show the *in vitro* activity of artemether, DHA or lumefantrine alone or in combination against the gametocytic and hepatic stages of *Plasmodium* species.

Activity *in vivo*

The activity *in vivo* was measured against the erythrocytic stages of *P. berghei*, *P. knowlesi* and *P. falciparum* strains in either mice or monkeys.

In studies performed in mice infected with *P. berghei* and treated at time of infection with lumefantrine or artemether showed a 50% reduction in parasitemia at doses of 1.27 mg/kg and 2.7 mg/kg respectively. The time required for reducing the parasitemia by 50% was 2 times faster in mice treated with artemether (mean, 23 hours) compared to that of lumefantrine (mean, 54 hours). Treatment with lumefantrine resulted in clearance of parasitemia, whereas treatment with artemether often resulted in recrudescence of infection. Drug combinations of artemether to lumefantrine in a ratio of 1:0.375 showed rapid reduction in parasitemia similar to that of artemether alone, and clearance of parasitemia similar to that of lumefantrine alone.

A study performed in monkeys infected with *P. knowlesi* erythrocytes and treated with artemether alone, showed a faster reduction in parasitemia. However, all the parasites were not cleared by day 105 in all animals. Treatment with lumefantrine alone showed a slower reduction in parasitemia. Recrudescence was reported in two thirds of the animals treated with the lower dose of lumefantrine

(12 mg/kg); all animals treated with the higher dose of lumefantrine (16 mg/kg), were aparasitemic on day 105. The drug combination of artemether and lumefantrine, irrespective of dose regimen used (either 1:4 or 1:6) was more effective in a faster reduction of parasitemia and clearance of parasites from blood in all animals than either drug alone. Similar results were observed in another study of monkeys infected with *P. falciparum.* No antagonism was reported.

Drug Resistance

In vitro studies in which the erythrocytic forms of *P. falciparum* K 1 strain were serially passaged (number of passages not indicated) showed no decrease in sensitivity to lumefantrine, artemether or the combination of artemether and lumefantrine.

In a study from French Guiana, the *in vitro* activity of artemether against *P. falciparum* clinical isolates, measured between 1997 and 2005, showed a decrease in the sensitivity to artemether in 2002 and 2005. Nine of the isolates in 2002 and 1 isolate in 2005 had an IC_{50} greater than 8.9 ng/mL. Molecular typing indicated that these isolates had a *PfATPase*6 –S769N mutant allele. Over a 3-week period, upon re-culture of the stored isolates with the mutant allele *PFATPase* 6-S769N and in the absence of artemether showed a decrease in IC_{50} value (1.42 ng/mL), suggesting a poor fitness of the mutant allele.

In vivo studies from mice infected with *P. berghei* strains showed that the potential to develop resistance to artemether is often unstable, resulting with the reversal to a more sensitive strain. The potential to develop resistance to lumefantrine alone is slow and resistance strains were observed after 7 – 11 passages in one study and 29 – 30 passages in another study. The potential to develop resistance using the combination of artemether and lumefantrine was similar to artemether in that it was unstable often resulting with the reversal to a more sensitive strain. Clinical relevance of such an effect is not known.

Cross Resistance

A positive correlation was observed in the *in vitro* activity between artemether and other artemisinin derivatives, halofantrine, mefloquine, amodiaquine, and negative correlation between artemisinin derivatives and chloroquine, suggesting variable cross-resistance depending on the region. In a study report from Africa, a positive correlation was observed between lumefantrine and artemether, pyronaridine, amodiaquine or quinine. The clinical significance of the *in vitro* cross-resistance observations is unclear.

Clinical Microbiology:
Cure rate in the clinical trials of Coartem was defined as the percentage of patients who cleared infection with no evidence of reappearance of the parasite within 28 days. Cure in this context was the absence of parasites based on microscopic examination of two consecutive negative peripheral blood smears. Some patients, although initially confirmed to have cleared parasites by microscopy, had reappearance of parasites within the 28-day trial window. These patients were regarded as treatment failures although the possibility remained that the reappearance of parasites following clearance was not due to recrudescence but a new infection. Methods exist for identification of distinct

strains/clones and have been used in epidemiological studies. However, the ability of these assays to distinguish a true recrudescence from a new infection has not been standardized and validated.

Two different techniques in two different laboratories were used to distinguish the genotype of the parasite(s) detected prior to treatment from those seen at reappearance. Samples from Studies A025, A026, and A028 were analyzed at the Shoklo Malaria Research Unit (SMRU) by a polymerase chain reaction (PCR) assay. Differences between genotypes were identified by allelic polymorphisms in 3 genes using 3 primer sets: merozoite surface protein-1 (MSP-1), MSP-2 and glutamate rich protein (GLURP). Each assay for each gene utilized a primary PCR with a second "nested" PCR on the product of the primary amplification. The differences between alleles were elucidated by comparing different PCR product lengths upon electrophoresis. The size of the PCR fragment was extrapolated by linear regression from a DNA ladder of known fragment sizes. The product was then sorted to a "bin" to classify the allele (see Table 45). For example, an isolate which had a PCR product of 450 bp for MSP-1, 552 bp for MSP-2 and 833 bp for GLURP would be categorized as a MSP-1 (bin 2), MSP-2 (bin 4), GLURP (bin 8) genotype.

Samples from Studies 2401, 2403 and 2303 were tested at the (b) (4) using a PCR and RFLP assay. For PCR, 2 primer sets were used (MSP-1 and MSP-2). Like at the SMRU laboratory, the assay for each gene utilized a primary PCR with a second "nested" PCR on the product of the primary amplification. Differences in MSP-2 were discerned using RFLP on the PCR products; if the baseline sample and that obtained at reappearance did not differ in genotype, then this was followed by analysis of MSP-1 which was clarified by differences in the length of the intact PCR products. A single difference in the genotype post-treatment compared to baseline resulted in the parasite being classified as a "new infection" and the patient was considered cured by the applicant.

The PCR and RFLP assays are considered experimental assays and have not been standardized and validated for the purpose of differentiating a new infection from recrudescence. The performance of these assays can vary from laboratory to laboratory. The performance characteristics of the assay were not submitted by the applicant for our review. Due to several limitations of the assay, results must be interpreted with caution. Some of these limitations were discussed by the applicant in the NDA submission in one of the study reports (Study 1003; for details *see Appendix*). Additionally, other potential confounders of this method have been identified.

1. **a) Sensitivity i.e. lower limit of detection of the assay**. Different lower limits of detection were used at different sites: SMRU used 1 parasite/μL, while (b) (4) used 25 parasites/μL and was demonstrated with gel results provided by the applicant. Variation in the limits of detection decreases the resolution of the genotyping procedure. Differences in the limits of detection between the 2 laboratories suggest that comparison across studies should not be conducted as the assays have not been standardized and validated.

   b) Sensitivity of the assays in infections with multiple strains of *P. falciparum*. Many of the baseline infections in the studies submitted by the applicant were due to co-infection with more than one strain or clone of *P. falciparum*. No assays were performed which allowed determination of the lower limit of detection in infections with mixed strains. The PCR assays have the capacity to identify and genotype mixed infections, though interpretation is often subjective. Factors which

may contribute to lowering the ability of the assay to consistently detect multiple strains/clones include primer bias, a disproportioned multiplicity of infection (MOI), and sequestration of one or more strains or clones of the parasites from the peripheral blood. These aspects of the assay are of critical importance since a strain missed at baseline would be improperly classified as a new infection if it is resistant to drug treatment.

2. **Specificity of the assay (human DNA or mixed infection with other species of *Plasmodium*).** The citation from which the primer sequences were taken stated that the assay is specific for *P. falciparum*. The applicant has stated that human DNA was run as a negative control on gels and results were discarded if the reaction yielded a product. However, the percentage of results which were discarded was not specified.

3. **Operator error and day to day variation in results (reproducibility and quality control).** No evidence was provided which showed reproducibility of the test results at each of the sites. Similar interpretation by at least two independent, experienced readers can provide assurance in the analysis of gel results. Gel results seem to have only been interpreted by a single reader. Additionally, the applicant has not submitted results of quality control testing.

   There is inherent variability in determining fragment length by linear regression which could result in misclassification of a genotype because of the "bin" procedure used at SMRU. For example, the estimated lengths of the GLURP fragment amplified from the baseline blood sample and the blood sample taken at parasite reappearance for patient 145 in Study 028 were 971 and 988 base pairs (bps), respectively. Therefore, the difference in estimated fragment lengths was 17 bp. Both of these fragments were allocated to bin 10. These results, along with the same bin classification of fragments produced for MSP-1 and MSP-2, resulted in this reappearance being classified as recrudescence. In another case however, for patient 296, a 17 bp difference was seen between the estimated fragment lengths for MSP-2. In this case, however, due to the cutoff between adjacent bins, the baseline product was categorized as bin 8 (710 bp) and the fragment amplified from the sample obtained at reappearance was categorized as bin 9 (727 bp). This difference led to the classification of this patient's reappearance as a new infection. Additionally, there may be bias in the assay for a high "false negative" rate due to operator and inherent day to day error (primarily misinterpretation of results, contamination or mislabeling). At SMRU, in order for paired isolates to be declared the same and thus the patient given the classification of recrudescent, 12 PCR reactions must yield identical products with no contamination or misinterpretation of results [2 samples (baseline & reappearance) x 3 genes (MSP-1, MSP-2, GLURP) x 2 PCRs (primary and nested)=12]. At (b) (4) there are 8 PCRs which must perform without error. A single episode of contamination, mislabeling or misinterpretation will yield incongruent results and the reappearing isolate would be identified as a new infection.

4. **Product confirmation (i.e., sequencing of PCR product).** Not reported by the applicant.

5. **Mislabeled and missing information from gel results.** Some of the actual gel results were provided and many were impossible to interpret due to unlabeled gel images and subject IDs which were not the same as in the trials. Additionally, some gel images were of very low quality.

Additionally, many elements of the above discussion are reflected in the study report for Study 1003. The author discussed potential confounders of the assay. Please see Appendix 1 for details. Some of the issues discussed above are also included in the World Health Organization consensus document[37]

The combination of artemether and lumefantrine (Coartem) is more effective than either drug alone as shown by studies conducted in China and Thailand. Additional clinical studies (comparative, non-comparative, or open-label) were done in Kenya, Nigeria, Tanzania, Mozambique, Mali, Republic of Benin as well as in non-immune travelers from Germany, Switzerland and Colombia. Coartem attains more rapid clearance of parasitemia than lumefantrine alone and is more effective in preventing recrudescence than artemether alone. Furthermore, the 6 dose regimen of Coartem was shown to attain higher cure rates than the 4 dose regimen. In comparative studies, Coartem was equivalent to MAS in its time to parasite clearance and achieved similar, albeit somewhat lower, overall cure rates.

The use of PCR assays to establish corrected cure rates was used in 6 of the 8 key studies. In most cases, the difference between corrected and uncorrected cure rates was negligible. However, in the two studies conducted in African children (2303 and 2403), there was a noticeable difference between uncorrected and PCR corrected cure rates. This is probably due to geographical location and trial design. The studies were conducted in areas of high transmission. The pediatric patients in both trials were not hospitalized or sequestered from potential infective mosquito bites during the trial. Several issues preclude the use of PCR genotyping for determining drug efficacy which include the consistency in detection of infections with more than one strain(s) and interpretation of results. In the absence of performance characteristics, quality control, standardization and validation of the assays, results of either PCR or RFLP assays should not be used for determining the efficacy of Coartem. Efficacy should be based on presence or absence of parasites on blood smears (i.e. uncorrected cure rates).

For mixed infections of *P. falciparum* and *P. vivax*, Coartem was effective in clearing the erythrocytic stage of both parasites (Table 85). However, due to the exo-erythrocytic hypnozoite stage of the *P. vivax* parasite, radical cure of *P. vivax* was not accomplished as recurrence or relapse was apparent in about one-third of the patients.

Table 85. Summary of patients who were co-infected with *P. vivax* and *P. falciparum* in Studies 25, 26, and 28**

Treatment Arm	Baseline			After initiation of treatment			
	Number of patients with *P. vivax*	***P. vivax* clearance time in hours [Mean (range)]**	**Number of Patients who relapsed**	**Number of patients with *P. vivax***	**Day of emergence [Median (range)]**	***P. vivax* clearance time* in hours [Mean (range)]**	**Number of patients who relapsed**
Coartem	28	21.5 (20.1 – 22.7)	9	32	17 (23 – 27)	142 (66.5 – 194.7)	1

The clearance time for patients with *P. vivax* emerging after baseline may be artificially high due to the infrequency of parasite counts during this period of the trial and some patients did not have confirmed clearance because they still had *P. vivax* parasitemia at the last visit.
** These were the only studies which reported data from which clearance time could be calculated.
# represents ranges of medians seen among the three studies and do not reflect individual patient data

Of the patients who had microscopically detectable gametocytes at baseline, during or following treatment, most patients treated with Coartem were shown to clear gametocytes as confirmed by microscopy. Results in Table 86 show that gametocyte clearance time, irrespective of the dosage regimens of Coartem, is less than 1 week for over half of the patients who presented with gametocytes at baseline. However, a number of patients that presented with gametocytes at baseline continued to remain positive for gametocytes through day 28 even though they were confirmed as cured for asexual parasites. The mean asexual PCT of those patients who had gametocytes does not differ from the overall mean PCT for each study. When comparing asexual PCT to gametocyte clearance time, it appears that asexual parasites are much more susceptible to drug treatment as the time required for gametocyte clearance is much greater than the time for asexual parasite clearance. Based on this data it is difficult to conclude whether Coartem is more effective in preventing gametocytogenesis or if Coartem is actually gametocytocidal. It may be that, given the fast action of Coartem and artemether, rather than being gametocytocidal, these drugs simply eliminate asexual parasites before they are able to differentiate to a sexual form. In a study conducted in pediatric patients by Bousema *et al.,*[40] the authors have concluded that microscope confirmed gametocyte clearance is not always an indicator of a patient being noninfectious as blood from those children with microscope confirmed gametocyte clearance could still infect mosquitoes. This could be due to the limit of detection of gametocytes and other limitations of the methodology. Quality control for gametocyte counts was available for review for only one of the studies in the NDA submission.

Table 86. Overall summary of gametocyte data from 8 key clinical studies, irrespective of dose and regimen of Coartem

Number of patients with gametocytes	**Gametocyte count at baseline or emergence [Median (range)]**	**Days to gametocyte clearance [Median (range)]***	**Hours to asexual parasite clearance [Median (range)]**
At baseline (n=108)	462 (124 – 3992)	5.9 (0.98 – 11.5)	34.7 (24 – 46)
After initiation of treatment (days 1 – 28) (n=124)	69 (4 – 123500†)	2.55 (0.75 – 13)	35.9 (30 – 44)

*It is important to note that the range for median days to parasite clearance is the range across studies and not for individual patients as some individuals never cleared their gametocytes from baseline to the end of the trial.
† The high end of the range for gametocyte counts seems abnormally high but was presented by the applicant in Study ABM02 (this number is the median of the gametocyte counts for two patients who presented with gametocytes after baseline in the Coartem arm)

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7. THE LABEL

7.1 Sponsor's Proposed Labeling

(b) (4)

[redacted]

7.2 Comments

1. The applicant has included the mechanism of action under section 12.1 and other microbiology information under section 12.2 of the package insert. The current format of antimicrobial labels has all microbiology information (mechanism of action, activity *in vitro* and *in vivo*, and drug resistance) included under section 12.4 with the heading "Microbiology". Therefore, the text should be rearranged to concur with the current content and format.

2. Section 12.1 "Mechanism of Action" should state "Coartem, a fixed dose combination of artemether and lumefantrine in the ratio of 1:6, is an antimalarial agent [see *Clinical Pharmacology, Microbiology* (12.4)]."

3. In the 'Mechanism of Action section, the applicant has described the chemical structure of artemether and lumefantrine. Such information may be described in section 11 "Description". Additionally, the applicant has included statements such as (b) (4) The statements in the labeling should be based on studies that were available for review. (b) (4) It is Division's policy to include only the activity of the molecule under review and not based on other molecules within the same class.

4. The applicant has proposed to state in the 'Activity *in vitro* and *in vivo*" section that (b) (4) Given the rapid action of artemether, rather than being gametocytocidal, the drug may simply eliminate early stage intra-erythrocytic sexual parasites before they are able to fully undergo gametocytogenesis while those mature gametocytes which were present at baseline succumb to senescence over time. The life span of a mature gametocyte has been estimated to range from 2.4 to 28 days[41]. This wide range of lifespan may account for gametocytes completely disappearing over time in some patients while gametocytes were still detectable in others at 28 days even if gametocytogenesis is abrogated.

   A study conducted in Thailand[42] demonstrated that artesunate reduced gametocyte carriage predominantly through a reduction in the number of patients who first presented with gametocytes following treatment thereby suggesting the effect of artesunate on the immature gametocytes. However, artemether was not included for testing. Several other citations from the literature support the ability of artemether to reduce gametocyte carriage over time[43-45] and suggest that artemisinin derivatives may be active against immature sequestered gametocytes but may not possess activity against circulating mature gametocytes. Specific gametocytocidal activity or reductions in gametocyte carriage relating to lumefantrine have not been demonstrated.

Quality control for gametocyte counts was available for only one clinical study (Study 2403) in the NDA submission and the results suggest difficulty in consistently being able to identify the gametocyte stages (see Table 72). Additionally, no effort was made to differentiate between different developmental forms of gametocytes.

(b) (4)

5. (b) (4)

7.3 FDA's version of the labeling

Based on the above comments, the following changes are proposed. Additions to the labeling are shown as double underlined and deletions are shown as strikeout.

12.1 Mechanism of Action

Coartem, a fixed dose combination of artemether and lumefantrine in the ratio of 1:6, is an antimalarial agent [see *Clinical Pharmacology* (12.4)].

(b) (4)

(b) (4) **12.4 Microbiology**

Mechanism of Action: Coartem, a fixed ratio of 1:6 parts of artemether and lumefantrine, respectively, is an antimalarial agent. Artemether is rapidly metabolized into an active metabolite dihydroartemisinin (DHA). The anti-malarial activity of artemether and DHA has been attributed to the endoperoxide moiety. The exact mechanism by which lumefantrine, exerts its anti-malarial effect is not well defined. Available data suggest lumefantrine inhibits the formation of β-hematin by forming a complex with hemin. Both artemether and lumefantrine were shown to inhibit nucleic acid and protein synthesis..

Activity In Vitro and In Vivo: Artemether and lumefantrine are active against the erythrocytic (b) (4) - stages of *Plasmodium falciparum.*

Drug Resistance: Strains of *P. falciparum* with a moderate decrease in susceptibility to lumefantrine alone can be selected *in vitro* or *in vivo* (b) (4) -The clinical relevance of such an effect is not known.

8. RECOMMENDATIONS

This NDA should be approved with respect to Microbiology pending an accepted version of the labeling.

_____	_____
Aaron Ruhland, PhD	Simone M. Shurland, MSc. PhD
Microbiologist, DSPTP	Microbiologist, DSPTP

CONCURRENCES:
DSPTP /Microbiology Team Leader _____Signature _____Date

CC:
DSPTP/Original NDA
DSPTP/PM/Gregory DiBernardo

9. APPENDIX

Rationale and limitation of the PCR analysis

The renewal of clinical activity (called recrudescence or re-infection in the analysis) when observed subsequent to treatment of a malaria case, and within an arbitrary 28 days period, is termed a clinical recrudescence. The success of the treatment as defined by the occurrence and timing of this clinical recrudescence (in association with regular microscopic examination of the blood during this 28 days period), serves to classify the parasites as sensitive (S) or increasingly resistant (RI-II) to the drug used. The clinical recrudescence is generally associated with both a rise in parasite numbers and with a concomitant return of malaria symptoms. In highly resistant cases (RIII) clinical symptoms and parasitaemia do not abate appreciably throughout the observation period.

Amongst the parameters used to assess the efficacy of a new treatment, the rate of clinical recrudescences is of some importance, as it reflects the effectiveness with which the drug removes and/or suppresses parasite growth. Clinical recrudescences are interpreted as resulting from parasite populations that survive the treatment in sufficient numbers to re-initiate a further symptomatic wave of parasitaemia. A problem in the interpretation of clinical trial results is encountered when the patient is still subject to bites by infective mosquitoes before, during and after treatment, as is the case in many endemic settings. Since the incubation period of *P. falciparum* can be as little as 8-10 days, the possibility exists that the renewed clinical activity following initiation of treatment results from a re-infection by a mosquito, rather than by survival of the any of the parasites present at the time of admission. Another overlooked possibility is the release into the blood, of parasites from the liver, which could have been inoculated by a mosquito before or during the first few days after initiation of treatment. A number of the drugs used to treat the blood stage parasites are not effective against the liver stages, and by the time the liver stage parasites mature and are released in the blood stream, drug concentrations might have reached sub-optimal doses.

P. falciparum parasites are known to be highly diverse. This diversity is reflected in the occurrence of many highly polymorphic proteins and the genes which encode them. In endemic regions extensive polymorphism of the parasite populations are frequently observed even in small villages of a few hundred inhabitants. Three *P. falciparum* genes which contain such polymorphic regions are MSP 1, MSP 2 and GLURP. When these three genes are used as genetic markers a genotype pattern for the parasite population present in a the sample analysed is obtained. Provided the relative frequency of the different allelic variants for the genetic markers is not highly skewed, and a sufficient number of variants for each of the markers is observed in a given area, there is low probability that infections initiated by different mosquitoes will result in parasite populations in the blood that share the same genotype pattern.

Therefore, the genotype pattern of the *P. falciparum* parasites present in the blood at the time of admission and during a renewal of clinical activity was obtained by PCR amplification of the polymorphic regions of the MSP 1, MSP 2 and GLURP genes. The genotype patterns of the two samples were then compared. This analysis was undertaken in order to ascertain whether it is possible to differentiate between genuine parasite recrudescences (survival of the drug treatment and therefore classified as a failure), and a case of re-infection (classified as a success).

It must be pointed out that the PCR analysis carried out in this study, and for that matter any other PCR analysis, will not and can not provide any conclusive evidence in favour of either alternatives (re-infection versus recrudescence). There are a number of reasons why this is the case:

1 - Parasites present at very low levels in the blood when the admission sample is taken are unlikely to be present in the aliquot analysed by PCR, and will not be therefore detected. These low levels could either be due to genuinely low parasite numbers for a particular population, or could result from the sequestration of mature parasites away from the peripheral vasculature. *P. falciparum* parasites are sequestered for the last 20 - 30 hours of their 48 hour erythrocytic cycle. Thus if two or more broods of parasites are present on alternate days (as is sometimes observed), or in asynchronous waves, it is quite possible to miss one parasite population when a single sample is analysed. Additionally, competition during the PCR amplification reaction, between two allelic variants of the same marker, might result in the detection of one but not the other.

2 - Parasites present in the liver during the early phases of treatment will not be detected by PCR analysis of blood samples.

The consequence of these two points is that detection of "novel" alleles in the renewal of clinical activity sample, could only be interpreted as circumstantial evidence that re-infection might have taken place.

3 - In cases where the genetic profiles are the same in the paired samples (or the pattern from the second sample represents a subset of the first), an interpretation of probable recrudescence of the original parasite population is acceptable. There is however, the possibility that the person acquires a new infection with a parasite profile similar to the admission sample. This cannot be excluded by simple frequency analysis of the alleles present, since mosquitoes can remain associated with a particular house (sheltering and feeding without leaving the dwelling), and the sporozoites they carry could retain their infectivity for 2 or more weeks.

Given that it is clear that no PCR analysis (as performed at present) can provide data for an unequivocal proof of a re-infection or recrudescence, the results of this study must be interpreted and conclusions derived bearing in mind the limitations described above.

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/s/

Aaron M Ruhland
11/25/2008 05:15:17 PM
MICROBIOLOGIST

Simone Shurland
11/25/2008 05:16:14 PM
MICROBIOLOGIST

Shukal Bala
11/25/2008 05:16:54 PM
MICROBIOLOGIST

MICROBIOLOGY FILING CHECKLIST FOR NDA or Supplement

NDA Number: 022-268 **Applicant:** Novartis **Stamp Date: 06/27/08**

Drug Name: Coartem (artemether and lumefantrine) **NDA Type:** Original

On **initial** overview of the NDA application for filing:

	Content Parameter	Yes	No	Comments
1	Is the microbiology information (preclinical/nonclinical and clinical) described in different sections of the NDA organized in a manner to allow substantive review to begin?	✓		
2	Is the microbiology information (preclinical/nonclinical and clinical) indexed, paginated and/or linked in a manner to allow substantive review to begin?	✓		
3	Is the microbiology information (preclinical/nonclinical and clinical) legible so that substantive review can begin?	✓		
4	On its face, has the applicant submitted *in vitro* data in necessary quantity, using necessary clinical and non-clinical strains/isolates, and using necessary numbers of approved current divisional standard of approvability of the submitted draft labeling?	✓		
5	Has the applicant submitted any required animal model studies necessary for approvability of the product based on the submitted draft labeling?	✓		
6	Has the applicant submitted all special/critical studies/data requested by the Division during pre-submission discussions?	✓		
7	Has the applicant submitted the clinical microbiology datasets in a format which intents to correlate baseline pathogen with clinical and microbiologic outcome?	✓		
8	Has the applicant submitted draft/proposed interpretive criteria/breakpoint along with quality control (QC) parameters and interpretive criteria, if applicable, in a manner consistent with contemporary standards, which attempt to correlate criteria with clinical results of NDA/BLA studies, and in a manner to allow substantive review to begin?			Not applicable.
9	Has the applicant submitted a clinical microbiology dataset in an appropriate/standardized format which intents to determine resistance development by correlating changes in the phenotype (such as *in vitro* susceptibility) and/or genotype (such as mutations) of the baseline pathogen with clinical and microbiologic outcome?		✓	The sponsor has used an experimental assay and the details provided were not complete. Additional information has been requested on the genotyping assay.

MICROBIOLOGY FILING CHECKLIST FOR NDA or Supplement

	Content Parameter	Yes	No	Comments
10	Has the applicant used standardized or nonstandardized methods for measuring microbiologic outcome? If nonstandardized methods were used, has the applicant included complete details of the method, the name of the laboratory where actual testing was done and performance characteristics of the assay in the laboratory where the actual testing was done?		✓	(see above) Actual results and performance characteristics have been requested for the genotyping assay
11	Has the applicant submitted draft labeling consistent with current regulation, divisional and Center policy, and the design of the development package?	✓		
12	Has the applicant submitted annotated microbiology draft labeling consistent with current divisional policy, and the design of the development package?		✓	More detailed annotations for microbiology section were requested
13	Have all the study reports, published articles, and other references been included and cross-referenced in the annotated draft labeling or summary section of the submission?	✓		
14	Are any study reports or published articles in a foreign language? If yes, has the translated version been included in the submission for review?	✓		

IS THE MICROBIOLOGY SECTION OF THE APPLICATION FILEABLE? ✓

If the NDA is not fileable from the microbiology perspective, state the reasons and provide comments to be sent to the Applicant.

Please identify and list any potential review issues to be forwarded to the Applicant for the 74-day letter.

Reviewing Microbiologist Date

Microbiology Team Leader Date

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/s/

Aaron M Ruhland
8/15/2008 10:18:08 AM
MICROBIOLOGIST

Shukal Bala
8/15/2008 10:28:22 AM
MICROBIOLOGIST